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Vol. LVI *May, 1929* *No. 5*

BIOLOGICAL BULLETIN

THE DIAGNOSIS OF MONOÖVULAR TWINNING.

II. Clinical Aspects.

HERBERT S. REICHLE, M.D.[1]

Since earliest times the occasional occurrence of extraordinarily similar twins has aroused the interest of mankind. The importance of this observation was however ignored by many centuries of scientists until Galton (1) in his researches into genetics in 1883 established the essential principles of our subject. Galton differentiated sharply between characteristics which are determined by the germ plasm and those which are developed by the interaction of organism and environment. He called attention to the existence of twins who showed remarkable resemblances and by refinements of differentiation he attempted to discover traits in them which were usually dissimilar. These differences, he suggested, defined the limits of inheritance. In them the individual play of chance or the effects of environment had exerted their influence.

The principles of modern genetics permit us to regard Galton's "identical" twins as products of the union of one egg and one spermatozoön. Evidence for this statement can be drawn from numerous sources. Obstetricians agree that the monochorionic twin is also a monoövular one. It is difficult to explain the large percentage of monochorionic births by any other embryological mechanism. Modern conceptions of teratology ascribe the formation of double monsters (cosmobia) to the incomplete division of two growth centers in the developing ovum. Weinberg (2) was the first to call attention to the statistical proof of monoövular

[1] From the Department of Pediatrics, Western Reserve University and the Babies' and Children's Hospital, Cleveland, Ohio.

twinning. Statistics from all over the world give the following ratio of sex correlation in twin births:

$$\frac{♂♂}{1} : \frac{♂♀}{1} : \frac{♀♀}{1}$$

Sex is however a Mendelian characteristic and should therefore be present in quite different proportions, namely:

$$\frac{♂♂}{1} : \frac{♂♀}{2} : \frac{♀♀}{1}$$

It follows that there are twice as many same sexed twins as there would be if sex selection were uninfluenced by other factors than Mendelism. This phenomenon is best explained by the assumption that about one half of all likesexed twins and about one third of all twins are monoövular. The latter are of course always likesexed. Observations from experimental biology (Loeb (3), Spemann (3) established the possibility of a monoövular development of two or more individuals and Newman's (4a) and Patterson's (5) work on the armadillo give us an almost complete history of the normal development of such individuals. The possibility that two individuals of different germ plasm can possess the striking resemblances often seen in so-called " identical " twins is, as Siemen has pointed out, infinitely small.

Such a phenomenon is of the greatest biological importance since it offers unique possibilities to the student of genetics. In such a pair of twins we have a clone, an identity in inheritance, which must of necessity be subjected to different environmental influences. We should be able therefore to discover the purely inherited factors of the human constitution and the degree to which these are modified by the environment; in the technical language of genetics we should be able to differentiate sharply between the genotype—the potential individual—and the phenotype—the actual individual. It should be possible to determine by a study of known monoövular and known diovular twins the acquired or inherited nature of any characteristic.

It is obvious that such a technique must be of immeasurable value to genetics. Its very promise however impels us to examine critically the premises upon which it is based. We must for the

present regard the fact that monoövular twins are the product of one sperm and one ovum as undebatable. We approach more debatable territory in our second assumption that such individuals possess identical germ plasms. The objections advanced against this assumption do not appear to us to be serious. Unless the chromosome division which precedes cell multiplication is equational—we except of course the maturation divisions which do not concern us in the discussion of our problem—Mendelism and in fact all our conceptions of cell inheritance must be worthless fictions. Such abnormal cell divisions do occur; but these exceptional cases do not destroy the universal validity of the biological law. Nature's mechanisms are rarely flawless. Idiokinesis—the phenomenon of unexplained chromosomal disturbance—is also a rare occurrence; moreover it has been observed chiefly in lower animals. We must also consider the possibility that the division of the egg into two independent units occurs later in its development, at a time when some division of cell potentialities has taken place. Characteristics would then have been segregated in the cells of the one individual and a difference in the phenotype would result. This is the one serious objection of all those that have been advanced.[1] Such an assumption is but ill supported by accepted biological observations. Serious—mostly lethal—results follow the separation of the developing organism into its constituent units at such a stage. Until more conclusive evidence is advanced we must accept the classical viewpoint that such a separation occurs very early in embryological development before segregation has taken place.

An article of Newman (4b) which appeared while we were correcting this paper accepts the view that segregation does take place. Newman believes that we can explain the varying similarity of monoövular twins by hypothecating a variation in the time period of the blastomeric separation. If this occurs after an axial asymmetry of the ovum has been established, important differences must appear in monoövular individuals. It is not the province of the author of this paper to discuss the possibilities of such a biological mechanism. It certainly does not appear that the

[1] Lenz (6), Newman (4) and Corner (7) are the principal proponents of this view.

biologists themselves are agreed upon the matter. We must however observe that if Newman's hypothesis is correct, the value of Siemen's technique for genetics must become very small indeed. It is therefore a very important question which Newman raises. The study of conjoined twins should aid us in answering this question for they—together with the double monsters—would in the light of Newman's hypothesis be the products of a late separation. They therefore should reveal greater dissimilarities than non-conjoined M. O. twins. We know of no facts which support such an assertion. More work along this line is indicated. It is however possible to explain the differences between monoövular twins in a manner quite different from Newman's, namely by the influence of early environmental changes in utero and after birth. We call attention to Schatz's (10) work, to the influence of the birth mechanism on the first twin born and to parakinetic factors which may differ for the twins during life. How incisive the latter may be has been described by Lange (*22*) in a very excellent article.

After we have satisfied outselves that our theoretical premises are sound, there still remains the eminently practical question—how shall we recognize monoövular twins? Evidently we must be very sure of this point before we can draw any legitimate conclusions from the use of our technique. The first attempt at a solution of this problem was made by Wilder (8) in his fundamental studies of palm and sole prints. We devoted our first paper to a discussion of their diagnostic value. We attempted to correlate similarity of palm and sole prints or the lack of this similarity with sex likeness in twins, as well as with the results of Siemens' diagnostic method. We also examined the palm and sole patterns of combined monsters (cosmobia), which are certainly monoövular twins. The three methods convinced us that dermatoglyphics [1] failed to give a clear and persuasive answer to our questions (*24*). Newman (4b) and Taku Komai (*23*) have arrived at different conclusions. However Taku Komai admits that he saw examples of discordancy between dermatoglyphics and other diagnostic signs of zygotism. We therefore do not

[1] Term coined by Cummins to include studies on sole, palm and finger prints.

believe that our disagreement with the Japanese writer is very serious. Newman on the other hand is very positive that dermatoglyphics is "the best single diagnostic aid." He says "a great deal of stress has been laid [by us] upon the diagnostic value of the palm and finger patterns. While this criterion is inadequate for certain diagnosis, it is surprising how few mistakes are made." Our experience with cosmobia and the many discordant results found in the literature do not permit us to share Newman's view. He might suggest and—if his theory of blastomeric separation be accepted—with right, that cosmobia are not the equivalents of his "identical" monoövular twins since they are the products of a separation occurring after axial asymmetry has been established. This would not decrease the value of our observations for as Newman himself will admit there is no essential difference between cosmobia and true monoövular twins. What is true for cosmobia, would therefore also be true for a certain percentage of monoövular twins.

The character of the birth membranes has always been considered the most significant sign of monoövular twin pregnancy. The relation between monozygotism and monochorionic membranes has been somewhat obscured by the misinterpreted observations of several prominent obstetricians. Strassmann (9) called attention to the fact that twins with monochorionic membranes usually show differences in weight and length that are greater than those of dichorionic twins. It is an error however to assume from this that monochorionic membranes are not associated with monoövularity or that such twins do not possess identical germ plasms. These differences are the greatest in early embryonic life (Schatz (10)) and grow smaller with the age of the fœtus. The lines of development continue to converge after birth as Verschuer's (12) figures prove conclusively. He found that the percentage difference of weight at birth was 7.8 for monoövular twins, whereas for diovular pairs it was only 6.3. For twins at the age of 19 years, however, these figures were 2.6 and 4.6 respectively. Schatz's work on the "third circulation" of the placenta in monochorionic twins explains this phenomenon. This painstaking worker showed that in such membranes there is an intimate anastomosis between the vascular systems of the

twins so that the blood from the one can flow freely into the circulatory system of the other. If through some trick of chance, the cardio-vascular mechanism of the one is superior to that of the other, the fortunate twin will gain an advantage in cellular nutrition. This leads to a hypertrophy of the one twin or to a hypotrophy of the other; in extreme cases the handicapped twin may develop hypoplasias of the cardiovascular apparatus which are incompatible with post-fœtal life or it may die in utero and degenerate into a fœtus papyraceous. After birth nutritional equality between the twins is restored and the genotypic identity may in the course of years eliminate these differences.

Siemens (11) has recently offered additional objections to the use of birth membranes in the diagnosis of monozygotism. He and Verschuer (12) have published observations on several individuals who showed an apparent conflict between the birth membrane diagnosis and the results obtained by comparing the bodily characteristics of the twins. We are not prepared to accept the radical view of these authors that monoövular twins may occasionally have dichorionic birth membranes, and diovular, monochorionic. The difficulties which embryology throws in the way of such an hypothesis are as yet too formidable to be overthrown by the evidence which results from the clinical study of twins. Despite decades of scientific obstetrics no case of monochorionic membranes in different sexed twins has yet been discovered. This is a crucial case which might establish Siemen's objections upon a legitimate basis.

The real difficulty of membrane diagnosis is the inadequate nature of the examination. In the majority of cases, of course, the results of the examination will not be available. When the reports are available, the investigator often discovers that the membranes have been examined solely to guard against retention of membrane parts and that no conclusion as to the nature of the twin pregnancy can be drawn. This at least was our experience. The description is usually summarized in a few words, such as: "Membranes entire, one placenta present." Of course dichorionic membranes may possess one placenta and it is probably possible for monochorionic membranes to have two. Equally unsatisfactory is a report which reads: "The twins are dichorionic."

This is a diagnosis and the investigator does not know the observations from which the conclusion has been drawn. The only report which can be safely used is one which describes the condition of the septum or which states that the two chorio-amniotic sacs were entirely separate. In monochorionic twins the septum should be composed of amnion only. Usually the two original layers cannot be separated. In rare cases even this sheet of tissue may be absorbed and the twins may lie in one cavity. In dichorionic twins the septum can usually be easily separated by blunt dissection into two amniotic layers, between which one or two chorionic layers are found. The answer to the question—monochorionic or dichorionic—is therefore given by an investigation of the septum. If the septum has been torn to shreds, it may be necessary to study microscopic sections.

One pair of our twins illustrates how misleading an incomplete report may be. Case No. 7 were unlikesexed twins, hence certainly diovular. They had dissimilar iris color, hair color, skin color, skin type, and dissimilar lanugo distribution. Yet the report of birth membrane examination is: "Placenta ovoid, 30 x 25 cm., two cords measuring 60 cm. each and inserting laterally. Single chorion with two amnions." It is evident that reports which neglect to describe the septum are valueless for the purposes of genetic study even though the records may have been made in a Class I Obstetrical Hospital, as was the case with us.

We must therefore agree with Siemens that the records of birth membranes will rarely be of value in differentiating monoövular from diovular twins. We do not however accept a diagnosis of monoövularity which is at variance with an authenticated membrane diagnosis. Cases of apparent conflict such as Siemens and Verschuer have described are not fit subjects for genetic research.

As can be deduced from the foregoing remarks, the diagnosis of monozygotism has hitherto been a difficult and an uncertain procedure. In 1924 Siemens (11) suggested an entirely new approach to this problem. He pointed out that monoövular twins must show a greater number of similarities in characteristics determined or modified by the germ plasm than diovular twins. Therefore twins possessing close similarities in essential characteristics are probably monoövular, the probability growing with the num-

ber of similarities. This assumption needs fear no statistical criticism; it is well known that in such a series the probability increases not arithmetically but geometrically. It is circumstantial evidence of the highest order and is the basis of the Bertillion and other like systems of individual identification. Several rules however must be observed. The characteristics which are chosen must be genetically unrelated;[1] otherwise their association in two individuals will constitute a spurious series. They must moreover be independent of environmental influence to a relatively high degree; we must therefore weigh very carefully the differences in the life histories of twins. It should not be forgotten that even in fœtal life remarkable parakinetic influences may develop. Thirdly, the characteristics must show a wide range of variation in the population from which the objects of the investigation are taken. For example, blue eyes, blond hair and florid complexion would be of little positive value in a Scandinavian country. The greater the heterogeneity of the population, the more valuable will be the comparison of similarities.

Siemens (11) selected the following characteristics for his system:

Group A. Traits "which are practically always exactly the same in the case of monoövular twins, and practically never so in the case of diovular." They are: Hair color and form, iris color, lanugo distribution.

Group B. Traits "which vary only slightly and rarely in monoövular twins but more so in diovular." They are: freckles, characteristics of skin vessels (cutis marmorata, telangiectasis, akroasphyxia), keratoses and folliculoses (ichthyosis, keratosis follicularis, acne), tongue furrowing.

Group C. Traits which "are usually alike in monoövular, rarely much alike in diovular twins." They are: face and head forms; ear, hand and nail forms, body build. To these Siemens adds the psychical attitude and inheritable diseases and malformations.

Other authors have suggested different methods. Schiff (14) and Wiechmann and Paal (15) have published articles on indi-

[1] Muller (13) believes that such a relation—linkage of the genes—is rare in man.

vidual blood grouping. Mayer-List and Hübener (16) have described the micro-capillary pictures of monoövular and diovular twins. Ganther and Rominger (17) presented new investigations in favor of the use of palm prints. Anthropometry has been exhaustively discussed by a number of writers: Schultz (18), Beckershaus (19), Siemens (11), Verschuer (12) and Dahlberg (20). Beckershaus (19) has compared the refractive indices of eyes and other data obtained by ophthalmometric technique.

Siemens (11), Verschuer (12), Dahlberg (20) and Newman (4b) have all published favorable reports of their use of the Siemens' method. The individuals examined by these authors were adults or older children. The present publication concerns itself with infants and younger children. This is an important difference, since there are a number of factors which make the application of the method more difficult in the younger subject. Perhaps the most important source of error is the delay in the manifestation of congenitally determined characteristics. The late development of the individual's permanent iris color is an example of such and it is easy to understand how fruitful of error the use of eye color would be if, as so frequently happens, the changes in the iris do not occur simultaneously in both twins. Many of the traits moreover do not appear until later in life. There is therefore a lack of differentiation in young children. The difficulty of all examinations at this age will also play a rôle. Lastly there is the important fact that monoövular twins will tend to converge in their lines of development, whereas diovular twins show divergence with their advancing years, as has been demonstrated above.

In order to avoid an artificial selection of our subjects, we examined all twins who were admitted to the Babies' and Children's Hospital and Dispensary of Cleveland. We also examined a series of twins who were born at the Maternity Hospital of Cleveland.[1] Our conclusions are drawn from the results of the examination of 38 pairs of twins whose ages ranged from new-born to 8 years. Four were new-borns and only four were over 5 years of age. Thirty pairs were likesexed; eight were unlike. Every

[1] We are indebted to Dr. Arthur H. Bill, Professor of Obstetrics and Gynecology, Western Reserve University for the permission to examine these infants.

precaution was taken to avoid any prejudice in diagnosing the type of twinning. No attempt was made to form a judgment of "identity" at the time of examination; the traits were noted on prepared blanks for each twin separately in a purely descriptive fashion. The similarities in the various traits were diagnosed many weeks later and an attempt was then made to diagnose the twinning by Siemen's method. This result was compared with the palm prints and, when it was available, with the birth membrane diagnosis. We have therefore avoided prejudice as far as possible. We made no attempt to obtain an exact mensuration of color and size since it was our intention to study the application of Siemen's method without burdening it with refinements which would hamper or nullify its routine clinical use. Verschuer (12) and Dahlberg (20) have used anthropometry with considerable success in the study of older subjects. However it seems to have only a statistical value at present since the average differences between monoövular and diovular twins are usually not large enough to exclude many cases which overlap.

The crucial test of Siemen's method must be our ability to demonstrate two groups of twins. The one must have identities in a large percentage of characteristics, the other must show a low percentage of identities. The members of the first group must be identical in the major characteristics of Siemens, namely, eye, hair and skin color and lanugo distribution, characteristics which Siemens together with other students of genetics regards as undoubtedly inherited. To these characteristics we must add sex; the members of the first group must always be alike in sex. In fact this is the one point about which there can be no argument. Monoövular twins must be likesexed.

We selected characteristics which were found in most of the twins of our group. These were:

Eyes	Sclera color
	Iris color
	Lashes, color and length
	Brows, color and length
Hair	Amount
	Distribution

	Color
	Texture and form
	Lanugo distribution
Skin	Color
	Type
Face	Form with detailed description of the various parts, such as brow, chin, malars, etc.
Mouth	Form
	Size
Teeth	Number
	Size
	Shape
	Character
Ears	Size
	Shape with detailed description of lobe, tragus, helix, etc.
Fingers	Size
	Shape

Temperament

Thirty-eight pairs of twins who had been adequately examined were utilized in our study. The percentage of identities in the above mentioned traits was computed for each pair. We expected to find the monoövular twins in the group with the higher percentage of identities. Our actual results do not support this hypothesis. It is true that the six cases with a birth membrane diagnosis which had been made by personal examination show no discrepancy between identity percentage and membrane diagnosis. On the other hand twins No. 30 which have a high identity percentage of 70 are unlikesexed, hence certainly diovular. Our expectation that this group of twins would be identical in the major traits of Siemens, iris, hair and skin color and lanugo distribution, was also unfulfilled. There are three pairs with high general identity percentages and notable discrepancies in the major traits. Twins No. 4 which have a general identity percentage of 64 have unlike skin color and lanugo distribution; twins No. 24 with a percentage of 77 have skin colors and lanugo distribu-

tions that vary in minor points, and twins No. 8 with a percentage of 78 have dissimilar irises. The case of twins No. 30 is however crucial. These twins had identities in all the major traits and the high general percentage of 70; yet they were unlikesexed and hence must be diovular. Cases 4, 24 and 30 were colored individuals. It is well known that variations in the darker shades of color are much more difficult to differentiate than those of the lighter. It is therefore possible that the high percentage of negroes in our group is in part responsible for the failure of Siemen's technique in our hands. This does not however explain all the discrepancies. In the entire series of cases—whether white or colored—there was a notable absence of that striking similarity between monoövular twins which one sees so frequently in the case of adults. This difference is probably due to the reasons which were enumerated above.

We must therefore conclude that Siemen's method is unreliable when applied to young children. We know of no other method which can replace it. We therefore do not believe that it is possible at the present time to diagnose with certainty the type of twinning when the subjects are of an immature age. Deductions drawn from such work are not above question. The results of our investigation do not of course apply to the use of the Siemen's technique when applied to older children or adults. Yet even within these limitations a sceptical attitude towards the method is still justified. There is perhaps at present a tendency to be overly anxious to reap the harvest from a technique which though very ingenious and promising has not yet passed out of the hypothetical stage. Despite the opinions of Siemens (11) and Newman (4b) we believe that more work must be done with *unselected* groups that include both like and unlikesexed twins. The absolutely determinate character of sex is too important in this field to be abandoned because of the complicating factor of sex linked genes. It is likely that such an investigation will substantiate Dahlberg's (20) opinion that Siemen's method is satisfactory if applied to a series of cases but that we cannot be absolutely certain of the diagnosis in an individual case.

After the completion of this study we discovered the publication of Klein (21). This author examined fourteen pairs of twins.

Only one pair was five years of age, the others were below two and one-half. In every case the birth membranes had evidently been carefully and adequately examined. Five dichorionic twins were identical in all of Siemens' twelve important traits; four other pairs showed great discrepancies between the birth membrane diagnosis and that made by the Siemen's method. Klein's work therefore substantiates our results.

Conclusions.

1. Our present methods of diagnosing the type of twinning are either impracticable or unreliable when applied to young children and infants.

2. Our most dependable source of information is the investigation of the birth membrane.

3. Reports of birth membrane examinations are valueless unless they describe the condition of the septum. It should be noted in the records of obstetrical institutions whether or not a layer of chorion was found between the two amniotic sheets.

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NOTES ON THE LOSS AND REGENERATION OF THE PELLICLE IN *BLEPHARISMA UNDULANS*.

J. ERNEST NADLER.

(From the Department of Physiology, University of Pennsylvania Medical School, Philadelphia.)

The color which is so characteristic of *Blepharisma undulans* has been the object of study and interest of many protozoölogists. It is usually a pink purple but may vary from deep purple violet to light rose, or the animals may be perfectly colorless. It has been recently suggested by Dawson (1929) that color changes accompany the process of digestion. It is believed that a somewhat different light is thrown on this general question by the observations here recorded.

When a drop of M/10,000 strychnine sulphate is added to individuals of this species on a slide they soon cast off their pellicles, swim away and leave the empty capsules behind. The color, so characteristic of this ciliate, is seen to be restricted to the pellicle which retains its color, the "naked" organism, on the other hand, being colorless.

During the process of shedding the pellicle, the cilia are withdrawn inside it where they go on beating in coördination (Fig. 1). This retraction starts at the anterior end of the animal and proceeds as a wave backward. After rotating for a short time inside of the pellicle the naked animal escapes by gradually working its way out in an ameboid-like manner through either the region of the posterior contractile vacuole or the base of the gullet. If for any reason the naked organism is unable to escape from the pellicle, death invariably follows. Dividing individuals shed their pellicle as one. Pieces of the ciliate, cut with a microdissection needle, are likewise able to shed their pellicles. Conjugating pairs shed their pellicles as do non-conjugnants but always escape through the gullet, either as separate individuals or as one, depending on the stage of conjugation and the degree of union.

During this process the conjugants frequently fuse giving rise to double monstrous forms.

The cast off pellicle is easily studied and presents a continuous

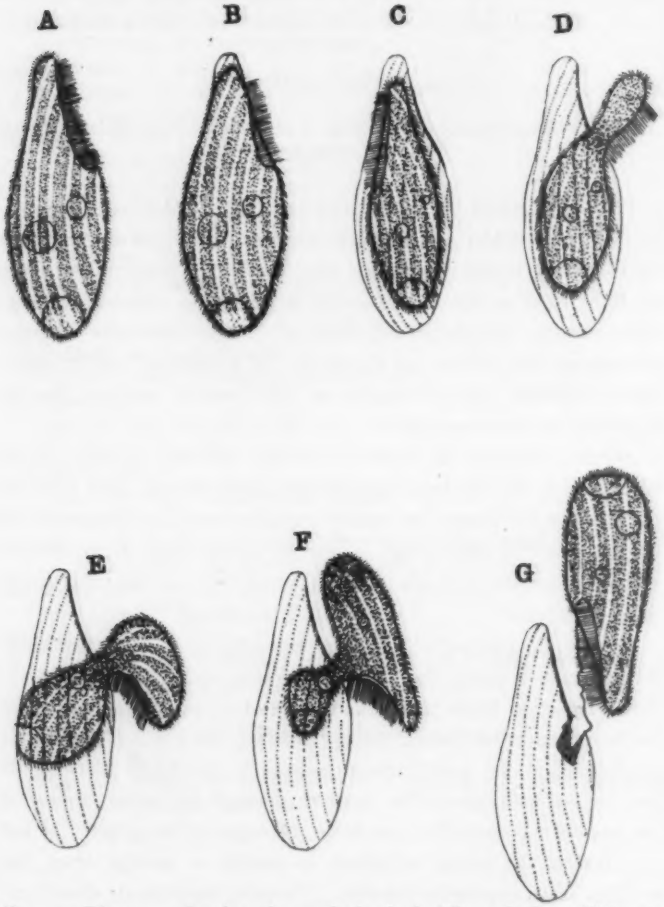

FIG. 1. Diagrams showing the method of shedding of the pellicle, the animal escaping by way of the gullet.

membrane-like structure with rows of small holes running lengthwise, through which the cilia had protruded, showing a definite arrangement of the cilia in from ten to eighteen rows. The

pellicle appears to be fastened more firmly at the base of the gullet than elsewhere and the empty membranous shell is usually dragged around by the animal for a short time by a strip of the pellicle attached to the base of the gullet.

The naked animal is much more flexible and is cut more easily with a microdissection needle than when the pellicle is intact. When cut, in this condition, the cut end instantly closes and the pieces behave like those with a pellicle.

The process of shedding may also be induced by a low concentration (M/100 to M/10,000) of morphine sulphate, codeine sulphate, cocaine hydrochloride and novocaine while it has not been found possible to produce it by the use of caffeine citrate, brucine, apomorphine, mercury succinate, picrotoxin, phenacetin, quinine hydrochloride, carbon tetrachloride, veronal, veratrine, nictotine, and saponin over a wide range of dilutions. Alcohol, ether and chloroform first decolorize and then dissolve the pellicle, both on the animal and when cast off. The chemicals that produce shedding do not seem to have any very obvious underlying property common to all, and little light is therefore thrown on the mechanism of the process. This fact is even more striking when the substances which produce shedding are compared with those which do not. The hydrogen ion concentration does not appear to play any great rôle in the process, as shedding can take place within the range of pH 5.6 to pH 8.2. Likewise, drastic alterations in the tonicity of the solutions by addition of salt or sugar do not cause shedding. This shedding is not merely due to a shrinking of the organism away from the pellicle, as caffeine produces such a shrinking without shedding of the pellicle taking place.

If *Blepharisma* is allowed to remain in a non-lethal concentration of the drugs which cause shedding, the pellicle after being lost is not regenerated. Individuals grown in M/100 morphine sulphate for 110 days did not form new pellicles although they grew, divided and moved perfectly normally. If removed to pure culture media as soon as the pellicle is shed the animals grow and divide, and after some time (1 to 12 days), regenerate new pellicles. Naked ex-conjugants may regenerate new pellicles in as short a time as 24 hours.

Regeneration is best obtained in culture media of wheat (average time for regeneration 9 days) and least successfully in hay infusion (average time 11 days, color pale). On a malted milk diet the pellicle is regenerated in about 10 days. The test for a new pellicle is the presence of color and the ability again to go through the shedding process.

Cultures with limited food supply are light pink in color while those with an excess of food are deeply colored. If starved, the organisms lose their color and pellicles in about 24 hours; the pellicle in this case does not appear to be shed but gradually becomes lighter in color and finally disappears (absorbed?). While the kind of food is likely an important factor in modifying the thickness of the pellicle the differences in color observed appear to depend to a marked degree on the thickness of the pellicle and this in turn on the amount of food available.

Further studies on the function and nature of the pellicle are in progress. It is a great pleasure to thank Professor M. H. Jacobs for many valuable suggestions.

Summary.

1. It has been observed that when *Blepharisma undulans* is treated with strychnine sulphate, morphine sulphate and several other chemicals the pellicle is shed. The pellicle and the "naked" animal have been studied.

2. The pink color so characteristic of this animal is restricted to the pellicle.

3. The pellicle is not essential for life, division or motion and its character is dependent on the amount of food available.

4. Under favorable conditions the pellicle is easily regenerated.

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RHEOTROPISM IN *UROSALPINX CINEREA* SAY.[1]

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Rheotropism has been observed in many animals (Schulze, 1870; Parker, 1903, 1908; Lyon, 1904; Jennings, 1904; Dimon, 1905; Hadley, 1906; Allee, 1912; Jordan, 1917; Arey and Crozier, 1919, 1921), and the receptors for this response appear to vary in the different species. Stahl (1884, from Verworn, 1899) and Verworn (1899) believed rheotaxis to be a positive response to pressure stimulation, a theory that was used by Wheeler (1899) to explain anemotropism as a special form of rheotropism. According to Schulze (1870) and Bonnier (1896) the reaction in fishes is brought about by the stimulation of the lateral line organs, an interpretation that was shown to be untenable for *Fundulus heteroclitus* (Parker, 1904) and for *Epinephelus striatus* Bloch (Jordan, 1917), where the organs of touch serve also as the essential organs of stimulation by water currents. Tullberg (1903) eliminated the ear of fishes and found that the animals operated upon were insensitive to water currents, from which he assumed that the ear was the receptor for this response. To this theory there are serious objections, as was pointed out by Parker (1903). A theory first proposed by Lyon (1904, 1909) and accepted by Loeb (1918) stated that in fishes "the primary cause of orientation in streams of some uniformity of motion is an optical reflex, a tendency on the part of the animals to follow the field of vision. . . . The essential element of stimulation is the environment not the current. . . . Contact between the fishes and stationary objects may lead to orientation. . . . In violent streams. . . . the fish may be oriented without sight or contact with solid objects. . . . here. . . . relative velocities constitute the essential elements of stimulation. If part of the water moves, and the next to it is

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relatively at rest, the fish may respond just as it does to contact with solids" (Lyon, 1904). "Fish with one eye blinded react to currents of water like normal fish. The usual form of stimulation is visual. The fish turn the nearest way to face the current, whether in so turning the motion be toward or from the injured side. . . . It seems to the writer impossible to bring these observations into accord with the tropism scheme of one-sided response to one-sided stimulation" (Lyon, 1909). Hadley (1906, 1908) showed that the lobster is rheotactic during its free swimming larval stage and by moving the environment rather than studying the animals in a current he showed that the optical stimulus alone is capable of producing this reaction. He adds that the rheotactic reaction *induced by currents* is more definite at night, tending to show that this is not optical. Main (1928) in the course of some experiments on the phototropism of fishes states "fish do not orient themselves in such fashion as to keep the *static* visual field the same."

In the literature on rheotropism there are no quantitative data available such as obtain for phototropism and geotropism. This may be due to the fact that the animals so far investigated have not permitted this type of study. For instance, it would be difficult to determine the rate of orientation of a fish in a stream of a given velocity. In a snail such as *Urosalpinx cinerea,* on the other hand, we have an animal that not only exhibits a precise and immediate reaction to water currents but is also admirably suited to quantitative study.

The following is a report of some observations on the effect of a current upon the movements of *Urosalpinx cinerea.* The interest of the data lies in the fact that this animal in its behavior to currents appears to follow Loeb's (1918) theory of tropistic conduct. Hitherto it has been impossible to interpret the reaction of animals to currents as a simple tropistic response (Lyon, 1909).

If *Urosalpinx cinerea* is placed in a current of water it orients itself so that the siphon is pointing upstream and then moves against the current. The response is definite and immediate. The removal of the eyes or of the tentacles does not disturb the precision and character of this reaction. Furthermore, light does not interfere with the orientation and movement, since experiments

carried on in the darkroom give results similar to those obtained in daylight. From these preliminary and general observations the work was expanded to include the following: (1) a study of the relation between the rate of current and the rate of creeping; and (2) a study of the relation between the rate of current and the rate of orientation (turning).

The apparatus used in these experiments consisted of a celluloid trough 2″ x 2″ x 20″, open at either end and suspended in a water current on an even keel so as to eliminate geotropic effects. Figure 1 gives two views of the apparatus and shows the direction of flow of the current. The lower view (1) shows a longitudinal half of the apparatus. The upper sketch (2) is a top view of the apparatus. The arrows indicate the direction of the flow of water in the various parts of the two troughs.

The rate of the water current was determined by noting the time necessary for uniformly-sized bits of cork to travel five inches. Fifteen to twenty readings were taken for each velocity. These were averaged and the figure thus obtained was used as the surface velocity of the current, from which the bottom velocity was determined (Gibson, 1925). Surface velocities from 1.25 to 7.60 cm. per second were used. The water temperature was kept constant by means of a thermostat, to within $\pm 2°$ Centigrade. The current was of the turbulent type, the only kind obtainable under these conditions.

The experimental animals were chosen for the definitiveness of their response alone, no attempt being made to obtain animals of the same size. Such selection is not only permissible but desirable (*cf.* Crozier, 1928). In all, twenty-five animals were used; eleven for the observations on the rate of turning, and fourteen for the experiments on the rate of creeping.

The data for the two series of experiments were collected in different ways. For the study of the relation between the rate of current flow and the rate of creeping, the time necessary for the animal to creep one half inch, directly against the current, was taken as a measure of the response. The procedure was as follows: after the desired current velocity had been obtained the animal was placed in the trough *B*. In a short while the animal would orient and begin creeping against the current. The time

(with a stop watch) necessary for it to creep one half inch was then noted. Since only those readings in which the animal crept actively and without apparent interruption were used, a mark on the shell was made to determine when the required distance had been traversed. Observations in which the animal pushed its shell forward without any actual translatory movement of the pedal mass were discarded. There were other factors which at times influenced the rate of creeping. For instance, the animal would sometimes veer off and strike the sides of the trough, and

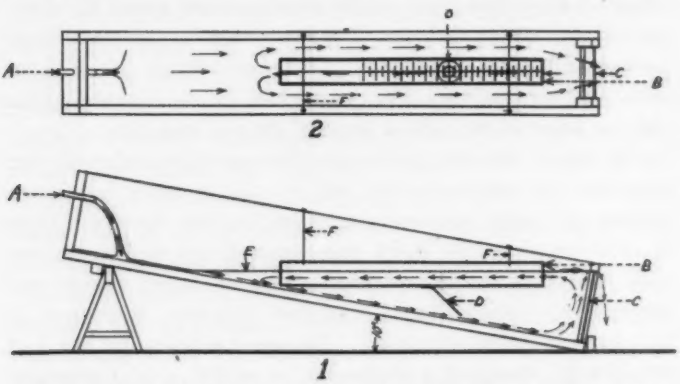

FIG. 1. Top (1) and side (2) views of apparatus. *A*, water inflow from coils placed in constant-temperature bath; *B*, celluloid experimental trough; *C*, glass front of outer tank; *D*, mirror at 45° angle to bottom of *B*; *E*, water level; *F*, wires supporting *B* on even keel; and *O*, system of circular and radial coördinates by which path taken by animal was copied.

occasionally a small mass of foreign substance resting on the bottom would interfere. All these records were disregarded. In a short while one could easily distinguish when the animal was moving actively and uninterruptedly. After each reading it was lifted from the substratum and the slime track cleaned away. In this way the influence of a previous track upon the movements of an animal was obviated. After approximately two to three minutes the next record was made. On the average ten readings were taken for each animal.

For these experiments fourteen animals were used, for which thirty-five satisfactory series of observations were made. In all

three hundred creeping rates at twenty-five different current rates were collected for the fourteen animals. Fig. 2 shows the results graphically when the current rate is plotted against the rate of creeping, both in inches per second.

Some slight but necessary modifications in procedure were made in the experiments on the effect of the current rate upon the rate of turning, but before giving these a word of explanation as to the apparatus is necessary. In Figure 1 (top view) there is seen the inner celluloid trough, in about the center of which are two concentric circles with perpendicular diameters. Immediately under this [as can be seen in the longitudinal section (2)] is placed a mirror (D) at an angle of 45° to the bottom of the trough. If one looks through the glass side (C) of the outer trough at the mirror (D) he sees reflected on the mirror the concentric circles (O). Thus when an animal is placed on "O," by

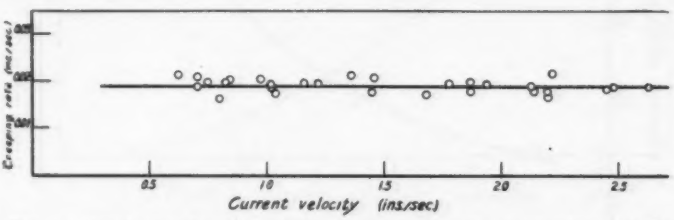

FIG. 2. Graph showing the relation between the rate of creeping and the surface current velocity both in inches per second.

looking through "C" at "D" one can observe the movements of its pedal surface. By the use of a similarly prepared record sheet the path of the animal is easily traced and a true record of the path of the animal's movements obtained.

The animal was placed at "O" and as soon as it began to creep and orient, its path was copied on the record sheet by observing continuously the path taken by a point on the pedal surface immediately behind the anterior transverse ridge, and a record was made of the time necessary for this movement. After each observation the slime track was removed. At least ten records were made for each animal. These were then measured for the total length of path and for the angular displacement of a tangent to the path, giving therefore three sets of figures for each trail: (1) the

total length of path; (2) the time required for such movements; and (3) the total number of degrees turned.

For these observations eleven animals were used at six different rates of current for a total of three hundred and two records. The results are shown in Fig. 3, where the square of the bottom

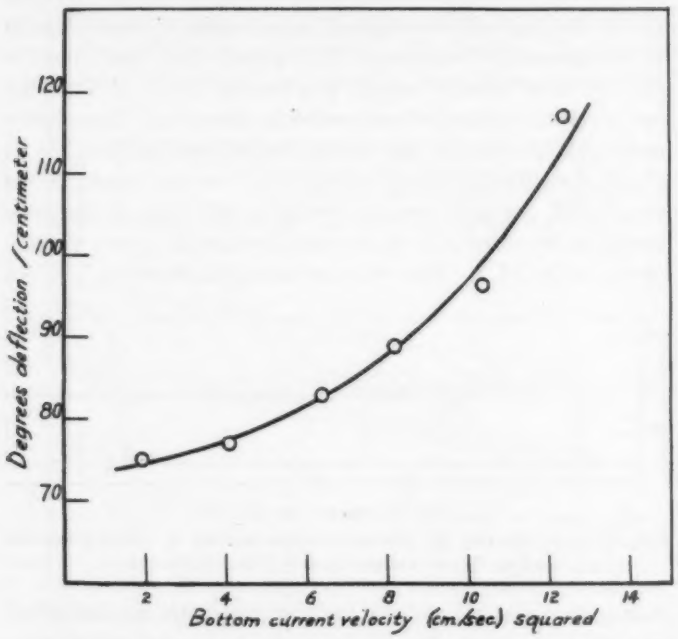

FIG. 3. Graph showing the relation between the bottom current velocity (cm./sec.) squared (Intensity) and the degrees deflection per centimeter of path (Effect).

current velocity is used as a measure of the intensity of stimulation and the degrees deflection per centimeter of path is a measure of the effect produced.

It is seen that the degrees turned per centimeter of path is a function of the rate of water current and that the curve (Fig. 3) is possibly the lower half of the S-shaped curve that one often obtains when the effect is plotted against the intensity (Hecht, 1922–23; Crozier, 1928). It was impossible to obtain points for

current rates higher than 7.60 cm. per second (at the surface) because above this the animal does not exhibit precise orientation and furthermore the stronger currents sometimes lift the animal from the substratum and passively wash it away. The receptors for this response seem to be the proprioreceptors located in the symmetrical parietal muscles. The unequal tension on these muscles, produced by the pull of the shell which in a stream tends to straighten out so that the shell presents the least resistance to the

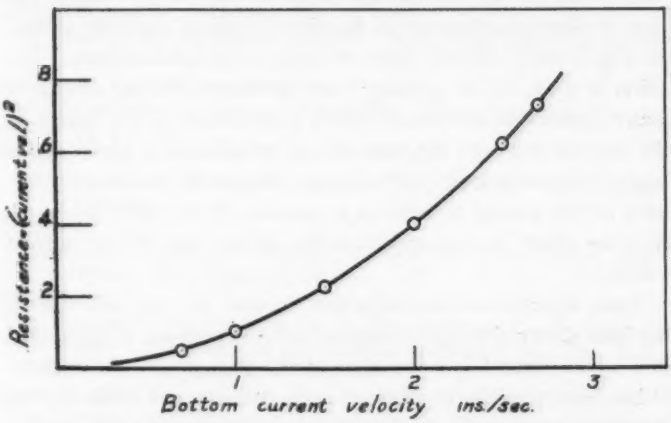

FIG. 4. Graph showing the relation between the bottom velocity in inches per second and the resistance overcome by the animal creeping against the current.

flow of water turning with the foot-mass as a pivot, is the stimulus which brings about orientation. This seems to be the same mechanism that functions in the geotropism of certain animals where it is proposed that the gravity responses depend on the stimulation of the proprioreceptors in the parietal elements (Cole, 1925–26; Crozier and Federighi, 1924–1925; Wolf, 1926–1927; Crozier, 1928).

Whether the mechanism of creeping in *Urosalpinx cinerea* is the ciliated epithelium of the pedal surface (Copeland, 1919, 1922), or whether creeping is due to "arhythmic" pedal waves (Parker, 1911; Crozier, 1919) it is evident that the rate of creeping is not affected by the velocity of the current (Fig. 2). It

is necessary however to note that although the rate of creeping is constant the resistance overcome is greater as the current-rate increases. Thus the animal must do more work as the velocity increases in order to maintain its uniform rate of progression since the pressure exerted by a flowing stream of water is proportional to approximately the square of the velocity (Gibson, 1925). In other words, since in the results all other factors remain constant and only the resistance overcome or the pressure exerted by the flowing stream varies as the current varies, this may be taken as a measure of the effect produced upon the animal. In Fig. 4 these derived data are given in graphical form. The effect is given as the pressure exerted by the flowing stream of water (resistance overcome) which is equivalent to the square of the current velocity; the intensity of stimulation is given as the bottom current velocity. This means that if the resistance overcome by the animal is taken as a measure of the effect produced, then the effect is proportional to the square root of the current velocity.

These conclusions are important because for the first time it has been shown that the orientation and the creeping of an animal in a water current is a function of the intensity of the current. It has been possible to measure both intensity and effect and to show that: (1) the rate of turning is a function of the current velocity, and if these are plotted there is obtained a curve which is similar to that obtained for other intensity *vs.* effect curves; and (2), the rate of creeping is independent of the current rate, but if one takes the resistance overcome rather than the rate of creeping as a measure of the effect then the effect is proportional to the square root of the current velocity.

Summary.

If *Urosalpinx cinerea* Say is placed in a current of water it will orient and move against the current. It has been possible to measure the rate of turning and the rate of creeping at various current rates. These results indicate that the rate of turning (degrees deflection per centimeter of path) is a function of the current velocity and that when plotted respectively as effect and intensity the curve obtained follows the usual effect *vs.* intensity curve obtained for other tropistic reactions. The organs of

stimulation for this response seem to be the proprioreceptors in the symmetrical parietal musculature of the animal. Although the rate of creeping is independent of the rate of current, the amount of resistance overcome (or the work done) is also a function of the current velocity.

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EXPERIMENTAL OBSERVATIONS UPON THE ENDODERMAL GLANDS OF PELMATOHYDRA OLIGACTIS [1]

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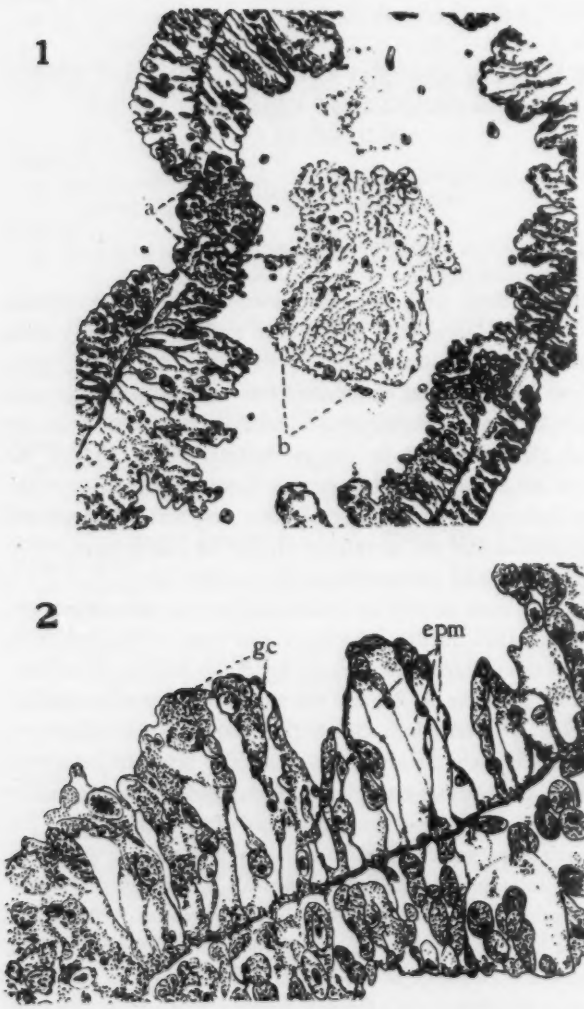
The species dealt with was determined to be *Pelmatohydra oligactis* (Pallas) according to Schulze ('17). This polyp has been under frequent observation in this laboratory for years. Kepner and Hopkins ('24) described two sets of glands present in the endoderm of *Pelmatohydra oligactis*. These are, first, the peristomal glands located in the peristomal region around the mouth, and second, the isolated secretory cells scattered throughout the endoderm. The presence of these two sets of glands led to the suggestion that the secretions of one set might be essential for the functioning of the secretions of the other set.

Experiments were devised to test the validity of this suggestion. Threlkeld and Hall, in this laboratory this year, determined that the range of the greatest tolerance of hydras to hydrogen ion concentration lay between pH 8.0 and pH 7.6, in other words upon the alkaline side of neutrality. Since the first phase of digestive processes in the lower invertebrates is usually acid, the inference was made that the peristomal glands discharged an acid secretion which activated the general secretory cells of the endoderm. To test this inference search was made for a microscopic organism that would tolerate an alkaline medium. After several trials, *Paramœcium caudatum* was selected. This organism will live for hours in the range of hydrogen ion concentration which represents the optimum for *Palmatohydra oligactis*. Having found this fact the following procedure was planned: Inject living paramœcia into two sets of polyps; one set having the peristomal glands and the other set having had them removed. To obtain the latter, normal hydras were placed under a binocular microscope and their

[1] These experiments were carried out at the suggestion of Dr. W. A. Kepner.



TEXT FIG. *A*.

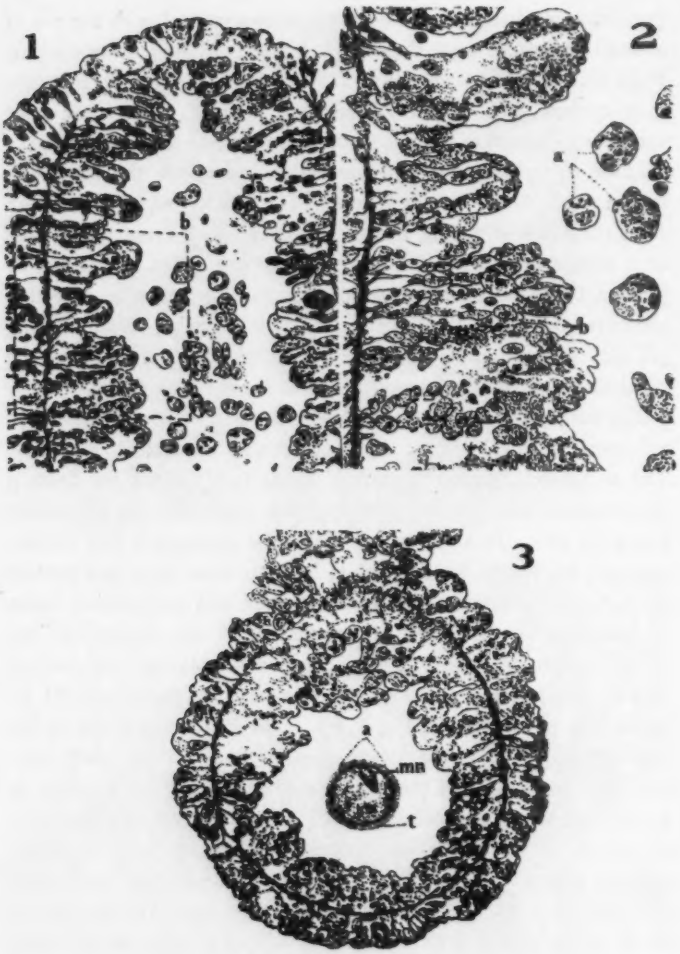
Explanation of Figures.

FIG. 1. Middle third of longitudinal section of complete polyp. *a*, wound made by pipette; *b*, remains of paramœcium 34 minutes after injection. ×750.

FIG. 2. Region of endoderm of complete polyp showing condition 34 minutes after injection of paramœcia. Gland cells (*gc*) stand out clearly in contrast with vacuolated epithelio-muscular cells. (*epm*). ×1500.

peristomal regions and the tentacles were removed with the aid of a small knife and were then allowed 8 to 10 hours to regenerate. Both sets of hydras were kept for 24 hours previous to injection in a covered dish in pH 7.8 solution without food. One hour previous to injection with food they were washed out with pH 8.0 solution by injecting it into the hydra just above the basal disc with a small, finely drawn pipette. The process of injection with food consisted of taking the paramœcia culture from the bottom of a centrifuge tube and mixing it with five times its volume of pH 8.2, being mixed in this proportion to insure that the injection solution would be well above neutral; the resulting mixture being pH 8.0. This mixture was also injected into the polyps above the basal discs and the hydras observed to see their reactions.

The first reaction of the complete hydra after such injection was to contract sharply, later contracting and expanding normally. The paramœcia, within the polyp, swam freely about for from 9 to 15 minutes and then began to appear in great distress, eventually becoming still. In all cases only after the paramœcia had become quiet did the hydras fix themselves by their basal discs and resume their normal positions. These were killed and sectioned in from 34 minutes to 1 hour and 40 minutes after the paramœcia had ceased swimming about. The sections when stained and studied showed paramœcia in various stages of having been digested by the hydras (Text Fig. *A*, Fig. 1, *b*). The endoderm of the hydra appeared normal and digestion appeared to be taking place normally. The sections of the complete hydras that were fixed 34 to 40 minutes after the introduction of the paramœcia into the body showed a perfectly normal endoderm. The epithelio-muscular cells are practically empty and highly vacuolated, as is usually the condition in a polyp from which food has been withheld for a period of 24 hours (text Fig. *A*, Fig. 2, *epm*). Not much of the material of the digested paramœcium had been absorbed during the relatively short period following the introduction of the ciliates into the cœlenteron. Another feature that is characteristic of the normal hydras is that the gland cells of the general endoderm stand out in sharp contrast with the epithelio-muscular cells (Text Fig. *A*, *gc*) in that they have inclusions that are peculiar to themselves. No such inclusions are to be found in the epithelio-



TEXT FIG. *B*.

FIG. 1. Longitudinal section of oral third of incomplete polyp 24 hours after removal of peristome and tentacles. Note absence of peristomal glands. Specimen fixed 5 hours and 10 minutes after injection with paramœcia. *a*, some of numerous endodermal cells that had migrated into cœlenteron; *b*, rectangle indicating region from which Fig. 2 has been taken. × 750.

FIG. 2. Region of endoderm of incomplete polyp 5 hours and 10 minutes after being injected with paramœcia. *a*, endodermal cells that had migrated

muscular cells. (Text Fig. *A*, Fig. 2, *epm.*) Another interesting feature of the histological picture presented by the complete polyps is the fact that in reacting to the introduced paramœcia few, if any, cells migrated from the epithelium of the endoderm into the cœlenteron (Text Fig. *A*).

The reactions of the hydras from which the peristomal glands had been removed were markedly different. Upon injection with paramœcia they expanded their fullest possible length and remained in this position for as much as twenty minutes. While in this position it was possible to see paramœcia swimming freely about in the cœlenteron. Eventually the hydras contracted to about one half their normal length. The paramœcia swam freely about and seemed to suffer no inconvenience from their close confinement. These hydras were observed for from 1 hour to 5 hours and eventually each of them egested the living paramœcia which swam freely away. Parenthetically, it may be of interest to record that not until the hydras had freed the paramœcia did they attach their basal discs to the substrata. This was done, however, by all of them after the paramœcia had been egested. It was interesting to observe that after having remained within these hydras for such long periods the paramœcia swam away in perfect condition. Not even their cilia appeared to have been eroded by digestive enzymes. But another observation was made that later proved to bring out, in sharp contrast, the reaction of the two sets of polyps upon which the experiment was performed. This observation was made upon many masses of refractive material which were thrown out of the cœlenteron together with the egested paramœcia from the incomplete hydras that had been injected with paramœcia. Upon examination under the 4 mm. objective, these masses appeared like minute conglomerations within which were many refractive bodies. In some cases an opaque, more or less centrally disposed body was seen. There

into cœlenteron; *b*, indicates the many inclusions to be found within the endoderm, which make it difficult to distinguish gland cells and epithelio-muscular cells. × 1500.

FIG. 3. Oblique transverse section through the basal third of polyp shown in Fig. 1. Observe transverse section of paramœcium (*a*) that shows no erosive effects of digestive enzymes. At the time the polyp was fixed paramœcium was alive, though 5 hours and 10 minutes had passed since it and many of its fellows had been injected into the polyp. *mn*, meganucleus; *t*, trichocysts. × 750.

were numerous such masses ejected in the case of each incomplete hydra. Their unstained condition suggested that they might have been general endodermal secretary cells that had left the epithelium of the endoderm.

These observations make the histological pictures of the incomplete hydras of interest. The histology of these hydras show that, in the first place, the introduced paramœcia had not been attacked by digestive enzymes. In one case, a paramœcium that had remained 5 hours and 10 minutes within the polyp and did not happen to be egested with its fellows was found in the sections. It proved to be well fixed, stained and sectioned. Even the trichocysts were clearly seen to be undischarged and lying within the ectoplasm (Text Fig. *B*, Fig. 3, *a*). Another conspicuous feature of the histology of these incomplete hydras is that there are numerous spheroidal inclusions within all the endodermal cells. These inclusions so much resemble those ordinarily encountered in the general gland cells of the endoderm and not ordinarily found in the epithelio-muscular cells, that one cannot here distinguish between general gland cells and epithelio-muscular cells with certainty (Text Fig. *B*, Fig. 2, *b*). Moreover, the histology of these polyps indicates that, though many cell-like bodies had been egested with the paramœcia, there yet remained numerous freed bodies which in the sectioned and stained material proved to be actually cells that had migrated from the epithelium of the endoderm (Text Fig. *B*, Fig. 1, *a* and Fig. 2, *a*).

SUMMARY.

It appears that the presence of peristomal cells are necessary for the killing and digestion of paramœcia when they are thrown into the cœlenteron that has been previously rinsed out with an alkaline medium. This raises the suggestion that the preliminary (acid) phase of digestion is induced by the secretions of the peristomal gland cells.

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OVULATION REQUIREMENTS OF *CULEX PIPIENS* LINN.

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It is very generally known that ovulation and oviposition in mosquitoes occurs after a blood meal. Females caught in their natural habitats or grown from larvæ will lay eggs within a few days or weeks after being fed upon an animal with blood. There seems to be a tendency among students of mosquito biology toward a tacit acceptance of the corollary that a blood meal is necessary to egg laying. Goeldi (1905) fed *Aëdes ægypti* on many substances such as fruit and honey and concluded that blood was necessary for ovulation in that species. It is altogether possible that such is the case with some of our species of blood-sucking mosquitoes. However, it will be shown in this paper that it is not true of one of our most domestic mosquitoes, *Culex pipiens*. There seems to me to be no *a priori* reasons for assuming that any species of mosquito should absolutely require blood in its diet before being able to ovulate and oviposit. Quite highly organized dipterous insects such as many of the muscoid flies, are able to lay eggs after feeding on such substances as sugar, honey, and milk. Most mosquitoes will imbibe many kinds of liquids and in nature they feed upon fruits and the nectar of flowers. One genus of mosquitoes, namely *Megarhinus*, has mouthparts of such construction that none of them can ever suck blood, and yet they persist in nature.

There are records of attempts to induce mosquitoes to lay eggs after meals other than blood some of which met with success. Ken (1917) working with *Aëdes scutellaris* was successful in getting eggs laid after meals of milk and sugar, peptone and sugar,

* National Research Fellow. This research was supported by a grant from the Wellington Fund. Thanks are extended to Dr. L. R. Cleveland for suggestions and aid.

and sugar only. This species failed to lay eggs after meals consisting of legumin and sugar and urea and sugar. In 42 experiments in which he fed milk and sugar, peptone and sugar, and sugar only, eggs were laid in 10 cases. Experiments by Fielding (1919) on *Aëdes ægypti* (*Stegomyia fusciata*) showed that eggs could be laid after diets of peptone and sugar but that all other foods used failed to bring about oviposition. These substances included sugar solutions, sugar and hæmoglobin, milk and sugar, banana, peptone solution, syrup, honey, dates, and apple.

In my own experiments I found no such elaborate technique as that employed by Ken (1917) necessary. The substances, in solution, were poured on absorbent cotton and this was placed on the gauze netting of the cage. When the mosquitoes had been kept away from food and water sufficiently long no difficulty was experienced in getting them to imbibe any of the liquids used. Some of the substances were offered each day for a week. This was not necessary in case of the richer foods such as serum and egg yolk. After 3 to 5 days the cages were placed over water. Oviposition occurred from 4 to 13 days after the first meal. The following table shows the results of experiments in which the larvæ had been fed upon high dilutions of milk and the resulting adults fed on the substances indicated.

TABLE I.

SHOWING THE RESULTS OBTAINED AFTER DIETS OF VARIOUS SUBSTANCES FED TO *Culex pipiens*.

Substance Fed	Result	Time Required	Viability
Peptonized milk	No ova		
Whey broth	No ova		
Whole milk	No ova		
Cabbage infusion	No ova		
Malt extract	No ova		
Raisins + haemoglobin	Ova	13 days	Hatched
Loeffler's blood serum	Ova	9, 10, & 11 days	Not viable
Haemoglobin solution	Ova	9 days	Hatched
Egg yolk	Ova	5 days	Hatched
Ox blood serum	Ova	6 days	Hatched
Ox gall	Ova	4 days	Hatched
Blood peptone	Ova	?	Hatched
Potato juice	Ova	6 days	Hatched
Carrot juice	Ova	5 days	Hatched
Apple juice	Ova	8 days	Hatched
Cell broth	Ova	7 days	Hatched

Some explanation about certain of these substances is desirable. The hæmoglobin was obtained from commercial hæmoglobin scales. Peptonized milk, whey broth, cabbage infusion, ox blood serum, ox gall, malt extract, and peptone were made from dehydrated products (Digestive Ferments Co.). Potato, carrot, and apple juice were expressed from the macerated vegetables or fruit, filtered, and fed undiluted to the mosquitoes. Cell broth was prepared by boiling a solution obtained from hæmoglobin scales and then using the clear supernatant fluid. (I am grateful to Dr. Cleveland for the method of preparing this medium.)

It will be noticed from these results that many substances may serve as adequate diets for the production of ova by *Culex pipiens*. These experiments were all done at least twice, with the same result each time. One exception not listed in the table was as follows. Ferric nitrate was added to milk to make a 10% solution. The filtrate was a clear, amber-colored liquid. In one experiment with this substance viable ova were obtained, but I was unable to get the same result upon repetition of the experiment.

Believing that the food of the larvæ might affect the ovulation of the adults I next tried experiments in which the larvæ were kept in a rich solution of hæmoglobin from the time of hatching from the egg to pupation. The adults from larvæ so grown were fed milk in one case and soaked raisins in another. In 5 days eggs were laid which hatched, and produced larvæ as vigorous in all appearances as those from ova laid under normal conditions. Larvæ grown in the routine way—that is, upon the bacteria and yeasts of cultures—have never produced adults capable of laying eggs upon a diet of milk or soaked raisins.

Gordon (1922) tried to determine what fraction of blood is necessary to ovulation in *Aëdes ægypti*. His summary is quoted "In a series of experiments in which fifty-four females were offered as food either serum, washed cells, or whole blood (the two latter being diluted with normal saline), it was found that the mosquitoes absorbed any of the fluids offered, but that oviposition only resulted in the case of whole blood." It is my opinion that the number of mosquitoes in each experiment was too small to permit us to conclude that whole blood is essential to ovulation in this species. It should be noted that *Culex pipiens*, as shown by

my experiments, is able to lay eggs after meals of blood serum, hæmoglobin, peptone, and cell broth, all of which are blood derivatives. In addition, they could ovulate and oviposit after meals of substances other than blood, including three substances of purely vegetable nature.*

These results lead me logically to conclude that *Culex pipiens* might persist very well in nature without having the opportunity to feed upon animals with blood. They also prove that there is nothing magical about the rôle of blood in the ovulation of this species.

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* Since this paper went to the Editor, I have observed one case in which a female laid viable eggs within 18 hours after emerging and *without having taken food of any kind*. About 50 eggs were laid and these produced larvæ of exceptional vigor as shown by the fact that they pupated on the fifth day after hatching. This experiment proves beyond any doubt that under optimum conditions the species could breed in the absence of many foods formerly considered essential to oviposition.

STUDY OF THE PHYSICAL PROPERTIES OF THE HEN'S EGGSHELL IN RELATION TO THE FUNCTION OF SHELL-SECRETORY GLANDS.

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A hen's eggshell plays an important rôle in the development of the embryo. It gives a physical protection; governs the embryonic respiration, serving as a membrane in the free interchange of gases; and it is also of value in the embryonic metabolism, notably in the mineral metabolism. For example, the calcium in the embryo largely comes from the mineral portion of the eggshell.

Besides its importance to the embryo, the eggshell has a great bearing on the food value of eggs. The physical condition and the perishability of an egg's contents largely depend upon the physical quality of the eggshell. Thus, a thin, a rough, or a cracked shell allows easy penetration by bacteria and molds, loss of moisture and carbon dioxide, and absorption of outside odors. At the same time such a shell breaks easily in handling or in transit.

All the above factors seem to have been recognized by both the scientists, in the fields of physiology and nutrition, and the practical men, in the fields of poultry production and marketing of eggs. Yet up to the present time very little work has been done on the determination of the physical properties of the eggshell. Among the workers to be mentioned here is Rizzo ('99). He, in the study of twelve hen's eggs, found that the number of pores per square millimeter of shell surface varied from 0.86 to 1.44 with an average of 1.23.

The present investigation concerns itself with the breaking strength, thickness, and porosity of the hen's eggshell, in relation to the function of the shell-secretory glands.

METHODS AND MATERIALS.

All the eggs used were from a flock of 91 White Leghorn pullets. During the experimental period of 16 weeks (from December 10, 1924, to March 31, 1925) the flock laid 3,998 normal

eggs. Production varied from 2 to 79 eggs per hen. The eggs were tested, the day that they were laid, for breaking strength and for thickness of eggshell. The breaking strength was measured by applying pressure to both ends of the egg in a specially constructed eggshell-testing machine (Fig. 1). The thickness was

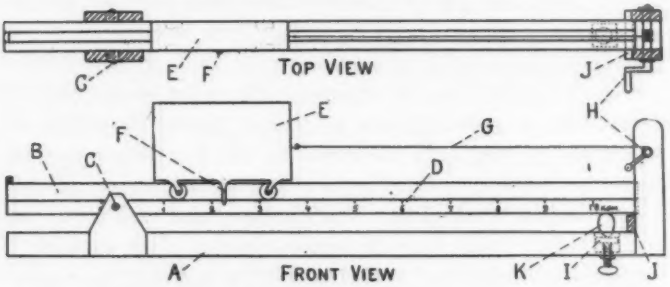

FIG. 1. A diagram of the eggshell-testing machine. *A*, frame; *B*, lever; *C*, fulcrum; *D*, scale; *E*, carriage; *F*, pointer; *G*, string; *H*, winch; *I*, adjusting stand; *J*, safety pin; *K*, egg.

measured by the micrometer caliper with ratched stop. Many eggs were also observed for the size and the location of pores on both the outer and inner surfaces of the eggshell.

RESULTS AND DISCUSSION.

The experimental data show that the breaking strength of the eggshell varies greatly not only among individual eggs but also among hens. The highest breaking strength was found to be 8.5 kilograms, while the average for 3,998 eggs was 4.46 kilograms. It was also determined that the average breaking strength of eggshell was less by one to two kilograms if the eggs were broken by applying pressure on the sides instead of the ends of the eggs. The tested eggs usually broke either on the blunt or on the pointed end, but very seldom on both ends at the same time. Of the whole number, 48 per cent. broke on the blunt end, and 52 per cent. on the pointed end.

The frequency distribution of variation in breaking strength is illustrated in Table I.

TABLE I.

FREQUENCY DISTRIBUTION OF VARIATION IN BREAKING STRENGTH OF EGGSHELL.

Breaking Strength (Kgm.).	Frequency Occurrence.		
	Number of Hens.	Number of Observations.	Number of Eggs per Hen.
2.01–2.40	1	2	2.0
2.41–2.80	1	37	37.0
2.81–3.20	3	117	39.0
3.21–3.40	7	224	32.0
3.41–4.00	9	329	36.6
4.01–4.40	20	848	42.4
4.41–4.80	23	1,097	47.6
4.81–5.20	18	858	47.7
5.21–5.60	8	439	54.9
5.61–6.00	1	47	47.0
Total	91	3,998	—

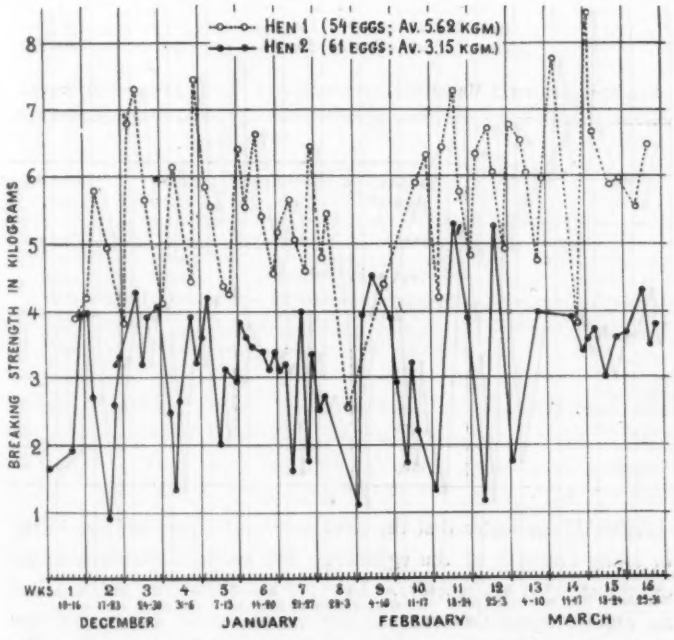

FIG. 2. Comparison of the breaking strengths of eggshell from two hens laying during the entire (16 weeks) period of observation.

The largest number of hens was found to be in the group averaging 4.4 to 4.8 kilograms. The number of eggs per hen, or the index of production, shows an increase with the strength of eggshell. In other words, the egg production stimulates the normal function of the reproductive organs and the eggshell secretory glands as well.

There is enough evidence to prove that the strength of eggshell varies with individuals. Figure 2 demonstrates a typical case, when two hens of almost equal production give a quite uneven average for the breaking strength of eggshell; the individual curves run distinctly apart throughout the observation period. The above figure and numerous observations by other individuals, show that the strength of eggshell is more uniform during a cycle of heavy egg production. Evidently, the secretary glands work normally at such times; and in practice, therefore, it would be advisable to select the hen for the strength of eggshell.

TABLE II.

RELATION BETWEEN BREAKING STRENGTH AND THICKNESS OF EGGSHELL.

Breaking Strength.	Thickness.			
	Broken End.	Unbroken End.	Blunt End.	Pointed End.
Kgm.	mm.	mm.	mm.	mm.
2.01–2.40	—	—	—	—
2.41–2.80	.246	.284	.290	.244
2.81–3.20	.259	.285	.287	.259
3.21–3.60	.274	.292	.291	.277
3.61–4.00	.287	.295	.292	.295
4.01–4.40	.302	.315	.312	.307
4.41–4.80	.325	.325	.325	.325
4.81–5.20	.333	.348	.333	.347
5.21–5.60	.343	.358	.343	.358
5.61–6.00	.356	.366	.345	.373
Average	.303	.319	.313	.309

Table II. indicates that the thickness is in direct relation to the breaking strength of the eggshell. The average thickness of all eggs was 0.311 millimeters. Eggs approaching this average had an almost equal thickness at both ends; while in general the broken end was thinner than the unbroken, and the blunt end was

thicker than the pointed. The pointed end, being the posterior part of the egg during its formation, gets comparatively less accumulation of the material in a weak and more in a strong eggshell.

The external structure of the eggshell, as seen under magnification (Fig. 3), suggests that a strong, thick eggshell has a large

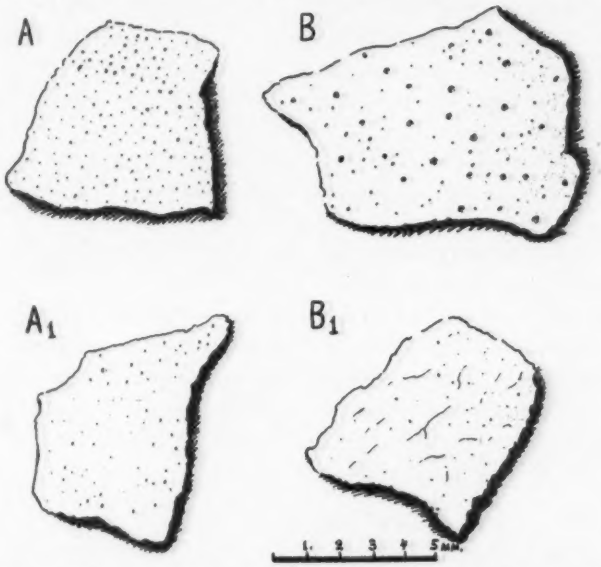

FIG. 3. Size and number of pores of eggshell as seen under magnification. *A*, outer, A_1, inner surface of a strong, thick eggshell. *B*, outer and B_1, inner surface of a weak, thin eggshell.

number of minute pores; while a weak, thin eggshell has few pores, but some of these are quite large in size. Besides, the inner surface of a weak eggshell has many grooves of various depths. Observation of the outer surface of the eggshell for the number and size of pores, may guide us in judging the breaking strength and thickness of the eggshell.

ACKNOWLEDGMENTS.

The eggs for this study were furnished by the Poultry Husbandry Department of Cornell University. To Dr. C. K. Powell,

of Cornell, I owe thanks for many suggestions, especially regarding apparatus and methods.

SUMMARY.

1. The data from 3,998 eggs show that the breaking strength and the thickness of eggshell are in the average 4.46 kilograms and 0.311 millimeters.

2. There exists a positive relation between the breaking strength and the thickness of an eggshell.

3. The breaking strength and the thickness of eggshell vary with individuals.

4. The variation of breaking strength and thickness of eggshell is the least at the time of heavy egg production. Therefore, the mean value of either the breaking strength or the thickness of eggshell may be easily determined by a few observations during the cycle of heavy egg production.

5. The porosity vary with the breaking strength and thickness of eggshell. The pores of the thick shell are small and numerous, while those of the thin shell are large and few in number.

6. The physical properties of eggshell presumably depend upon the individual function of the secretory glands during egg formation more than any other external factors.

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EFFECT OF EXCESSIVE DOSAGES OF THYROID ON THE DOMESTIC FOWL.*

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In attempting an analysis of the character of barring in poultry its physiological basis was studied. Since barring is due to the rhythmic deposition of black pigment and since other workers who fed the thyroid gland of cattle to poultry found that the pigmentation of the feathers was affected, it was decided to study the effect of feeding thyroid to Barred Plymouth Rocks.

The thyroid, which is one of the endocrine or ductless glands, is located in the fowl on the ventral side of the common carotid artery at a point where it touches the jugular vein. It is a small, oval, red body with a fibrous capsule. There are two small parathyroid bodies attached to the lower pole of the thyroid.

OTHER WORK.

Much work has been done with the treatment of various species of animals with thyroid. However, the work with the domestic fowl is somewhat limited. The earliest work was done by C. J. and C. Parhon (1914), who fed dry thyroid powder every other day (.15 grams) to 6 pullets. Marked excitability resulted, with tremors and ischemia or local anemia of the comb. Five of the thyroid-fed pullets were at the end of a year exposed to cholera, 2 surviving (40 percent. survival). Of nine similar control pullets one survived (11.11 percent. survival). The authors were led to conclude that "this confirms the rôle of the thyroid gland in the production of immunity."

* Published with the approval of the Director of the Kentucky Agricultural Experiment Station. The investigations reported were initiated November, 1924, at the Kentucky Experiment Station. From September, 1925, to June, 1926, they were continued at the Wisconsin Agricultural Experiment Station, and subsequently completed at the Kentucky Agricultural Experiment Station. Published with the approval of the Director of the Wisconsin Agricultural Experiment Station.

Torrey and Horning (1925) suggested "a certain antagonism between ovary and thyroid in connection with pigment formation that has no counterpart in the male." Crew and Huxley (1923), with daily feeding of 2 grams of desiccated thyroid per chick from 3 months to 7 months of age, to R. I. Reds or Light Sussex males, failed to observe the assumption of hen-feathering noted by Horning and Torrey.

Giacomini (1924) fed raw ox thyroid to fowls, starting with pieces the size of a hemp seed, gradually increasing the dosages until in some cases as much as 5 grams was fed daily. He observed depigmentation and a "profound stimulating and accelerating action of the thyroid hormones" upon basal metabolism, especially catabolism. Zavadovsky (1925a) fed excessive dosages, which were highly toxic and caused a precipitous molt followed by striking depigmentation in the new feather growth. Zavadovsky (1925b) notes that with single dosages of 30 to 50 grams of desiccated thyroid gland there was a complete fall of the feathers by the seventh to fourteenth day, followed by a new growth of plumage by the 21st to 30th day. He attributed the striking change in plumage to the specific action of the thyroid on the pigment-forming mechanism. Horning and Torrey (1927) criticize this explanation and in referring to the excessive dosages as highly toxic state that the striking change in plumage is "induced by an excessive, essentially toxic dosage of thyroid rather than a specific action of the latter (thyroid) on the pigment-forming mechanism." In all their work they used non-toxic dosages, usually 1 gram of desiccated thyroid per 5,000 grams of body weight of the fowl. With such dosages they were able to maintain the health of the hens, which in turn also laid hatchable eggs.

Method of Procedure.

In order to study the effect of large dosages of desiccated thyroid on barred plumage, hens, cockerels and capons were fed single doses varying from 10 grams to 35 grams. On January 9, 1926, ten Barred Plymouth Rock hens (hatched April, 1925) from the production-bred strain kept at the University of Wisconsin, were penned separately and fed thyroid.[1] Since large dosages

[1] Armour's desiccated ox thyroid, U. S. P. 0.2 per cent. Iodine.

were fed it was thought best to add it to the mash. In several cases the hens did not eat all the mash so the remaining portion was weighed back and the approximate consumption of thyroid computed. The birds were weighed at the start of the test and observed for condition of molt. The feathers loosened and a molt occurred in all hens fed 10 or more grams.

General Results on Hens.

In all cases, thyroid administration was followed by an increased nervousness and activity. Plymouth Rock hens, normally of a rather gentle or phlegmatic disposition soon changed to a highly nervous condition. They resembled more the Leghorn in nervous temperament and activity, indicating the possibility of a breed difference in basal metabolism, having its seat in the thyroid gland. The thyroid-fed hens developed a high, shrill voice uncommon to Barred Plymouth Rocks.

Among the eight hens which survived thyroid administration seven showed a loosening of the feathers, which pulled out quite easily from 4 to 9 days later. A rather precipitous molt followed in these 7 hens in from 7 to 10 days (see figure 1). Careful

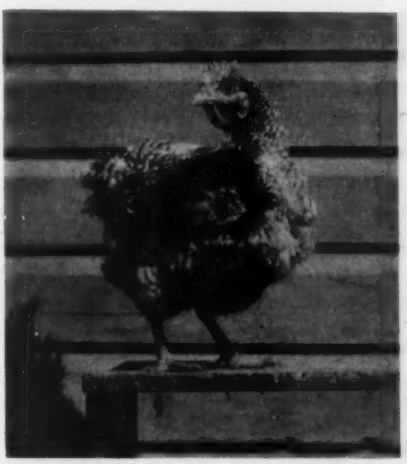

Fig. 1. Hen 4101 showing molt induced by single heavy dose of thyroid: fed 20–22 gms. thyroid Jan. 24, 1926; photographed Apr. 5, 1926.

records of the rate of molt were kept for hens 112 and 152. The feathers apparently loosen by the fourth day after thyroid administration and a molt follows by the seventh day, reaching its height by the eighth to eleventh day and then slackening rather abruptly by the fourteenth day, after which only a few occasional feathers fall. It is interesting to note that the incoming feather germs

TABLE I.

SHOWING DOSAGES FED HENS AND EFFECT ON FEATHERS.

Band Number.	Amount of Thyroid.	Days Elapsing Until		
		Feathers Loosen.	Body Molt.	White Appears.
57.....	8 to 9 Grams	No molt		None
2355.....	12 to 14 Grams	9	9	None
3670.....	10 Grams	8	10	None
4101.....	20 to 22 Grams	5	9	30
3930.....	23 to 25 Grams	Died Five Days Later		
47.....	15 Grams	No molt		
	15 Grams [1]	Died One Day Later		
110.....	20 to 25 Grams	Loose at start	9	30
119.....	15 to 18 Grams	8	10	44
152.....	8 to 9 Grams	No molt		None
	24 Grams [2]	4	7	40
112.....	0 Grams	Not loose	None	None
(Control)....	30 Grams [2]	4	8	40

take several days longer to loosen the primary and secondary wing feathers than the body feathers. This precipitous type of molt is not uncommon to heavy layers which molt late (October and November).

The fact that such sudden and precipitous molts do occur in late-molting heavy-laying hens under normal conditions and that molt was not precipitated when 50 mg. of sodium arsenite was fed to 3 different Leghorn pullets (75 mg. proving lethal) tends to indicate that the striking molt was due to a hyperthyroid condition, speeding up abnormally the basal metabolism, rather than to the toxic effect of larger than physiological doses.

[1] Additional dose fed 7 days later.

[2] Fed in capsules 30 days after negative treatment noted above.

Feather Renewal.

In five of the seven hens in which molt occurred there was noticeable depigmentation in the new feathers. All seven hens showed a silky nature of the feathers growing in after thyroid feeding. Horning and Torrey (1923) noted "a corresponding in-

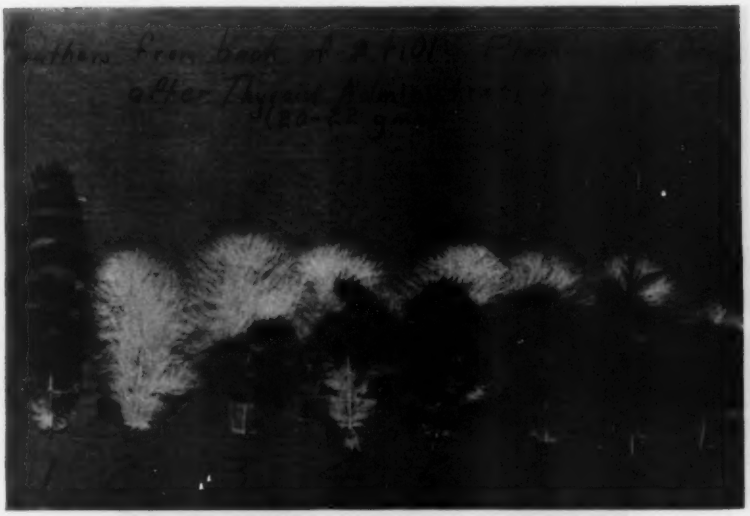

Fig. 2. Effect of thyroid administration on feather structure and pigmentation.

crease in the number and distribution of the barbules on the barbs" following thyroid feeding. Later, these authors (Horning and Torrey, 1927) report that "as a rule, this pigment is carried by the barbules and limited by their distribution."

Figure 2 shows feathers taken hen 4101 (fed 20 to 22 grams) after renewal of the feathers subsequent to thyroid feeding. Feather No. 1 shows the normal plumage, since it is a feather that was not molted. Feathers 2 to 7 show increasing amounts of pigmentation. All 8 feathers were taken from the back of Hen 4101, illustrated in Fig. 1. The heavy condition of molt of the hen is noticeable in Fig. 1 (Note the similarity to a rapid molting high producer). The photographs of the hen were taken 70 days

after thyroid administration. Feathers 2 to 5 (Fig. 2) are completely silky in appearance, there being on observation with the naked eye no apparent interlocking of barbules on adjacent barbs as illustrated and described by Lloyd-Jones (1915). Feathers 6 and 7 show a slight interlocking close to the shaft at its distal end.

FIG. 3. Hen 4101 showing depigmented feathers under left wing (also abundant under right wing and on back). After subsequent molt, feathers in these same areas were normally barred when observed, Aug. 30, 1926.

Feather 8 had obviously commenced its growth prior to thyroid feeding as the distal end, including the first three black bars and two white bars, is normal in every respect (the portions missing in feathers 1 and 8 were removed for microscopical examination). The abnormally wide white bar in feather 8 resulted from thyroid feeding. This white bar and the remaining proximal portion of the feather are of the silky nature indicating the absence of interlocking barbules. A microscopical examination reveals the absence of the hooked hæmules of the barbules in the lower silky-

appearing portion of the feather and their presence in the distal (normal appearing) end. This is illustrated in Fig. 4. The "silky" barbule has essentially the same appearance as in the silky fowl. This similarity indicates the possibility that the mechanism causing the presence of silky feathers in the silky fowl has its seat

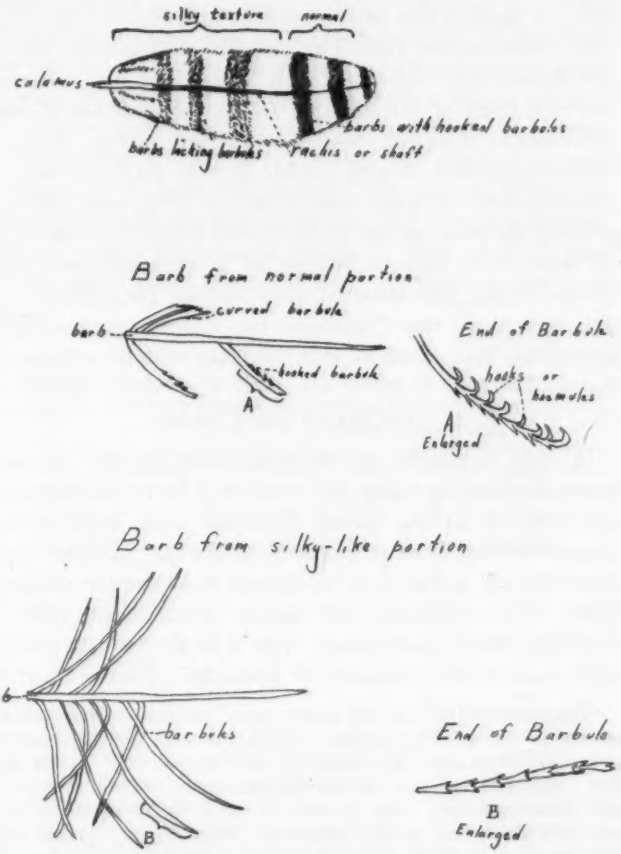

FIG. 4. Feather No. 8 from ♀ 4101. (See Fig. 2.)

in the thyroid gland. Perhaps a hormone from the thyroid gland in this breed, transmitted to each feather germ through the blood

stream, has an inhibitory effect on the barbules thereby preventing the development of hooks (hæmules).[1]

It is noticeable from a glance at Figs. 2 and 3 that feathers being renewed at the same time have black pigment (melanin) deposited in differing amounts. While pigment was being deposited in rhythmic waves in feathers 5, 6 and 7, resulting in well-defined bars, no pigment was being deposited in feather 2 and numerous other white feathers (note Fig. 3). This indicates that the rhythmic deposition of pigment in each feather, resulting in barring, is not centralized or synchronized for all feathers, but is rather a phenomenon separately controlled by each feather germ. This independent effect on each feather germ is further borne out by observations of normally molting hens in which some feathers are growing the black portion of the feather while others are growing the white. The author's findings are in agreement with those of Torrey (1926), who observed when studying the rhythmic deposition of pigment that "whatever the underlying mechanism, its activity has been associated experimentally with the activity of the thyroid."

Cockerels and Capons.

In order to observe the interrelationship, if any, between the hormones from the testes and the thyroid hormones, both capons and cockerels of the Barred Plymouth Rock breed were fed dosages varying from 10 grams to 35 grams. In order to check accurately the amount fed, the thyroid was placed in gelatin capsules.[1] This experiment was started December 22, 1926. The stock was March hatched and from a strain bred the preceding eight years at the University of Kentucky. When two or more

[1] The paper by Danforth and Foster (1929), on skin transplantations, appearing in *Jour. of Exp. Zoology*, Vol. 52, No. 3, pp. 443–470, came to the author's attention after this manuscript had been submitted. Their findings that "With the Silkie . . . the determining factors for both color and texture (hookless barbs) seem to reside in the follicles themselves" do not lend apparent support to this supposition. However, both findings indicate that certain genes in the feather germ for a character are expressed with the aid of hormones circulating through the blood stream to the feather during its development.

[1] Quarter-ounce capsules, each containing 5 grams of thyroid, were used. The large end of the capsule was dipped in cod liver oil before administration to aid in the passage through the gullet and the crop.

doses were fed they were administered on succeeding days. Ten cockerels and ten capons were given doses as indicated in Table II. Two controls were kept of both capons and cockerels.

It should be noted that 2 capons and 1 cockerel died after administration of thyroid. The temperature of the capons was extremely high just prior to death (111½° and 116° F.) The birds were down on their legs, giving the appearance of paralysis, and they died in convulsions and tremors. Post-mortem examination revealed in both cases contracted ventricles, dilated auricles and an excessive amount of straw-colored fluid in the pericardial sac. The anterior lobe of the left kidney was enlarged in each case. Edema of the lungs was marked. The cockerel showed no adverse symptoms the evening before his death. A post-mortem examination showed appearances similar to those of the capons except that only a small amount of fluid was found in the pericardial sac.

TABLE II.

SHOWING DOSAGES FED MALES AND EFFECT ON FEATHERS.

Coop.	Amount of Thyroid.	Days Elapsing Until		
		Feathers Loosen.	Slight Molt.	Heavy Molt.
Capons				
1.....	Control	Not loose	No molt	No molt
2.....	10 Grams (1) [1]	9	9	23
3.....	15 Grams (1)	6	9	10
4.....	15 Grams (1)	6	10	23
5.....	20 Grams (1)	3	3	9
6.....	20 Grams (2)	6	10	Not heavy
7.....	25 Grams (2)	6	9	10
8.....	25 Grams (5)	6	9	10
9.....	30 Grams (2)	3	9	10
10.....	30 Grams (3)	3		(Died—5 days)
11.....	35 Grams (2)	3	3	(Died—5 days)
12.....	Control	Not loose	No molt	No molt
Cockerels				
21.....	Control	Not loose	No molt	No molt
22.....	10 Grams (1)	10	10	Not heavy
23.....	15 Grams (1)	6	9	Not heavy
24.....	15 Grams (1)	9	10	10
25.....	20 Grams (1)	6	10	Not heavy
26.....	20 Grams (2)	6	10	Not heavy
27.....	25 Grams (2)	6	7	10
28.....	25 Grams (5)	6		(Died—7 days)
29.....	30 Grams (2)	6	10	13
30.....	30 Grams (3)	6	9	10
31.....	35 Grams (2)	6	9	10
32.....	Control	Not loose	No molt	No molt

[1] Total amount divided into number of doses indicated in parenthesis.

Feather Changes.

Areas of the saddle and back were plucked at the time of thyroid administration in order to check on the new feathers grown. In addition to the growth of feathers in the plucked areas, new feathers grew to replace those molted. In structure and depigmentation the new feathers resembled those discussed in detail in the case of the hens. However, the depigmentation was not nearly so marked in the males.

Effect on Weight.

All the males were weighed at the time of thyroid administration and at stated intervals thereafter. At the same time, body temperature was taken by inserting the bulb of a clinical thermometer well into the vent. Work of Fronda (1921) shows that the body temperature of the fowl is nearest the average or normal from 4 to 6 P.M. and 8 to 10 A.M., hence the temperatures were

Table III.

Body Weight of Males.

Coop.	Weight at Start.	Gain [1] at 48 hrs.	Gain [1] at 5 days.	Gain [1] at 7 days.	Gain [1] at 12 days.	Gain [1] at 31 days.
Capons						
1 (Control)	6 lbs. 12 oz.	0 oz.	−2 oz.	−4 oz.	+1 oz.	+4 oz.
2	6 lbs. 15 oz.	−3	−9	−9	−4	+5
3	7 lbs. 12 oz.	−11	−15	−12	−11	−3
4	7 lbs. 8 oz.	−8	−9	−4	0	+2
5	7 lbs. 15 oz.	−6	−10	−11	−3	+1
6	8 lbs. 15 oz.	−9	−14	−7	−5	+5
7	7 lbs. 2 oz.	−4	−18	−21	−9	−2
8	7 lbs. 2 oz.	−1	−6	−14	−11	+3
9	8 lbs. 8 oz.	−6	−12	−15	−9	−3
10	6 lbs. 12 oz.	−8	−12	Dead		
11	8 lbs. 4 oz.	−4	−13	Dead		
12 (Control)	7 lbs. 0 oz.	+4	+4	+2	+7	+8
Cockerels						
21 (Control)	7 lbs. 12 oz.	+5	+1	+3	+8	+9
22	8 lbs. 12 oz.	−4	−7	−4	+1	+8
23	8 lbs. 0 oz.	−1	−1	0	+4	+16
24	7 lbs. 8 oz.	−4	−11	−17	−8	+3
25	7 lbs. 10 oz.	−2	−5	−1	+2	+17
26	8 lbs. 8 oz.	−1	−11	−19	−11	−3
27	7 lbs. 14 oz.	−3	−18	−9	−7	+2
28	8 lbs. 6 oz.	−1	−6	−20	Dead	
29	7 lbs. 15 oz.	−3	−11	−14	−13	+1
30	8 lbs. 9 oz.	−3	−5	−7	−4	+1
31	7 lbs. 11 oz.	−3	−11	−7	−3	+5
32 (Control)	7 lbs. 9 oz.	+8	+7	+9	+11	+19

[1] Based on weight at start.

taken at those times. Table III. shows the effect of thyroid administration on weight and Table IV. the effect on temperature. The average weight of the four controls was 7.26 pounds, while

TABLE IV.

TEMPERATURE OF MALES.

Coop.	Temperature at Start.	Decrease [1] at 48 hrs.	Decrease [1] at 5 Days.	Decrease [1] at 7 Days.
Capons				
1 (Control)	107 1/5° F.	0.2° F.	0.2° F.	0.2° F.
2	106 4/5°	1.4°	0.2°	0.4°
3	106 2/5°	1.2°	0.8°	0.0°
4	106 4/5°	2.4°	0.4°	0.4°
5	106 4/5°	1.4°	0.4°	0.4°
6	106 2/5°	0.8°	0.4°	0.4°
7	106 2/5°	1.4°	1.6°	0.4°
8	106 4/5°	1.2°	2.2°	2.0°
9	106°	0.8°	0.0°	0.6°
10	107 4/5°	2.4°	3.7°	Dead
11	106 4/5°	1.6°	9.2°	Dead
12 (Control)	106 3/5°	0.0°	0.6°	0.2°
Average of treated birds		1.46°	1.95°	0.57°
Average of controls		0.1°	0.4°	0.2°
Cockerels				
21 (Controls)	107 3/5°	0.6°	1.0°	0.2°
22	106 4/5°	1.8°	0.2°	0.2°
23	106 4/5°	1.0°	0.0°	0.4°
24	106 4/5°	1.2°	0.4°	0.4°
25	107°	0.8°	0.0°	0.4°
26	106 2/5°	0.2°	0.0°	0.2°
27	106 4/5	1.6°	0.0°	0.6°
28	106 3/5°	0.8°	0.4°	Dead
29	106 3/5°	0.8°	0.2°	0.4°
30	106 2/5°	1.0°	0.6°	0.0°
31	106 2/5°	0.6°	0.2°	0.4°
32 (Control)	106 2/5°	0.4°	0.4°	0.4°
Average of treated birds		0.82°	0.2°	0.33°
Average of controls		0.5°	0.7°	0.3°

that of the 20 thyroid-fed males was 7.88 pounds, hence it may be seen that the birds to be fed thyroid had a slight advantage in size at the start of the test. At the expiration of 48 hours after administration of the first dose, all thyroid-fed males had lost weight (varying from 1 to 11 ounces per bird) whereas no loss of weight appeared in the controls. In 19 of the 20 thyroid-fed males loss in weight increased from the 2d to the 5th day. By

[1] Based on temperature at start, capon temperatures taken from 4 to 6 P.M., and cockerel temperatures taken from 8 to 10 A.M.

the 7th day 9 of these had regained some of the loss in weight. The capons averaged a loss of 6 ounces each by the second day, whereas the cockerels lost only 2.5 ounces. By the 5th day the capons had lost an average of 11.8 ounces each and the cockerels 8.6 ounces each. When compared with the controls it may readily be seen that a decided loss in weight occurred subsequent to thyroid administration, the loss being more pronounced in the capons than in the cockerels. This loss in weight was found by Giacomini (1924) to follow thyroid administration in larger than physiological doses, and was attributed by him to the stimulus given to basal metabolism (especially catabolism), rendering such birds unable to utilize properly the carbohydrates in the feed. However, he reported no difference in the effect on capons and cocks, whereas the writer found indications of a greater disturbance, occurring more quickly, with the capons, substantiated by the greater drop in temperature. Perhaps this may be due to a lack of compensatory hormones from the testes.

Effect on Temperature.

The average temperature of the controls was 106.95° F., and of the birds to be fed thyroid 106.68° F. at the beginning of the test. Thyroid administration resulted in a significant decrease of the body temperature. Forty-eight hours after giving the first dose the average temperature of the controls was 106.65° F. whereas that of the 20 birds fed thyroid was 105.54° (1.11 degrees lower than controls). The average decrease for the capons was 1.46°, while for the cockerels it was only 0.82°.

Summary.

1. Single doses of desiccated thyroid ranging from 8 to 30 grams although producing physiological shock were not lethal to hens.

2. Single doses of 30 to 35 grams proved lethal to two out of three capons.

3. Cockerels are able to withstand single dosages as large as 35 grams.

4. Single doses varying from 8 to 35 grams cause a loosening

of the feathers in from 3 to 10 days. Molt commences in from 3 to 10 days.

5. Depigmentation occurs during feather renewal following the precipitous molt. It was quite noticeable in 30 days after feeding.

6. A silky texture to the feathers was noticeable following thyroid feeding.

7. The hooks or hæmules are absent from the barbs in the feathers growing in immediately following thyroid feeding in larger than physiological dosages.

8. Thyroid feeding in large doses causes a change in nervous temperament of the Plymouth Rock, making it highly excitable.

9. Loss in body weight follows feeding of thyroid in large dosages, more especially in capons.

10. Thyroid feeding has a depressing effect on body temperature when fed in large dosages, capons showing a greater depression than cockerels.

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THE EFFECT OF AMMONIUM SALTS ON PROTOPLASM OF AMŒBA.

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There has been a vast amount of work done on the effect of chemicals on plant and animal cells. The methods employed in studying this effect have been largely confined to immersion of the cells in solutions of the various reagents. It is well known that immersed cells may be affected in a number of ways: the reagent may act on the plasma membrane, may affect the internal protoplasm without injuring the membrane or it may affect both the membrane and the internal protoplasm. Until the advent of the micropipette (Barber, 1911; Kite, 1915; Chambers, 1922) it was not possible to ascertain how the substances affect or react with a cell. The work of Chambers (1926), Reznikoff (1926), Pollack (1927), Hiller (1927) and others have brought to light, by microinjection studies, many facts concerning the differences between the plasma membrane and the internal protoplasm and their reactions with various chemicals. Many substances such as narcotics, carbon dioxide, hydrogen cyanide, hydrogen sulfide, picric acid and certain salts which are lethal to immersed cells (amœbæ) have been found to be only reversibly injurious when injected into the cell. The action of strong acids and bases has been found (Chambers and Reznikoff, 1926) to be largely confined to the surface of the cell. HCl, pH 3, and NaOH, pH 9, when injected into amœba do not irreversibly injure the internal protoplasm, while amœbæ immersed in these solutions die very quickly.

From the work of Harvey (1911), Jacobs (1920) and others it appears that strong acids and bases enter living cells very slowly whereas weak acids and alkalies penetrate cells with little if any resistance. It has been rather generally accepted that the toxicity

[1] National Research Fellow 1927–1928. The author wishes to express his appreciation to Dr. C. E. McClung, University of Pennsylvania, for the use of a laboratory during the tenure of the fellowship.

of the weak acids and bases is due to their ease of penetration. However, the question of whether their toxicity is due to the effect on the plasma membrane or on the internal protoplasm has not been satisfactorily answered. Recently it has been shown that CO_2 (Chambers and Reznikoff, 1926), HCN and H_2S (Brinley, 1927 and 1928) when injected into amœba do not kill the cell unless the dosage is so large that it ruptures the plasma membrane. On the other hand, amœba die very quickly when immersed in these solutions. These experiments seem to prove conclusively that CO_2, HCN, and H_2S exert their lethal action primarily on the cell membrane and not on the internal protoplasm.

In view of the fact that ammonia enters cells very rapidly, in this respect being similar to the weak acids, it was thought desirable to ascertain whether the toxicity of ammonium hydroxide and other ammonium salts was due to their action on the plasma membrane or on the internal protoplasm. The present paper deals with the effect of certain ammonium salts, namely, hydroxide, chloride, citrate, phosphate and acetate on the protoplasm of amœba as determined by immersion and injection experiments.

Immersion Experiments.

Amœbæ were immersed in solutions of the following ammonium salts: hydrate, chloride, citrate, phosphate and acetate, and their effects on the organisms were studied. The species of amœba was not determined but it undoubtedly belonged to the proteous group. The concentrations of the solutions were N/10 and N/100. No attempt was made to control the H ion concentration but the pH was determined in each case by the colorimetric method. The reaction of the amœba to each salt will be discussed separately.

Ammonium hydroxide: Amœbæ immersed in N/10 ammonium hydroxide (pH 9.8) withdrew their pseudopodia and assumed a spherical form. The plasma membrane ruptured within a few seconds and the cell disintegrated. When amœbæ are immersed in N/100 solution the cell assumes a spherical form and swells slightly. Brownian movement becomes very rapid. The cell membrane dissolves within 3 to 5 minutes and the fluid protoplasm disperses into the surrounding solution.

Ammonium chloride: Amœbæ immersed in N/10 NH_4Cl, pH 6, elongate into the limax form and continue locomotion at a reduced rate for over an hour. The viscosity of the protoplasm seems to be slightly increased. At the end of two hours locomotion ceases, the animal rounds up and the cell disintegrates. When amœbæ are immersed in N/100 solution they assume the limax form and locomotion continues for over eighteen hours.

Ammonium carbonate: Immersion of amœbæ in N/10 or N/100 $(NH_4)_2CO_3$ results in an immediate cessation of locomotion, the pseudopodia remain extended and there appears to be a slowing down in the rate of Brownian movement. The cells swell slightly and the granules collect near the center of the cell and the protoplasm coagulates.

Ammonium acetate: Amœbæ placed in N/10 acetate solution continue to move at a slow rate for two hours. Finally the cell assumes a spherical form and disintegrates within two or three hours. They remain alive and continue locomotion in N/100 solution for over eighteen hours.

Ammonium citrate: Amœbæ immersed in N/10 or N/100 citrate solution elongate into the limax form and resume locomotion. The cell finally rounds up and the protoplasm coagulates.

Ammonium phosphate: Amœbæ placed in N/10 or N/100 phosphate solution ($NH_4H_2PO_4$) continue locomotion for several hours. The streaming of the protoplasm becomes sluggish and finally the protoplasm coagulates and the animal dies.

TABLE I.

THE COMPARATIVE TOXICITY OF CERTAIN AMMONIUM SALTS TO *Amœba proteus* (?).

Salt.	Concentration.	Time Required to Kill 75 per cent of the Organisms.	pH
Hydroxide	N/10	15 to 30 seconds	9.8
	N/100	3 to 5 minutes	
Carbonate	N/10	1 hour	8.5
	N/100	1.5 hours	
Citrate	N/10	5 to 10 minutes	5.4?
	N/100	3.5 hours	
Phosphate	N/10	2 hours	6.8
	N/100	4 hours	
Chloride	N/10	2 hours	5.0
	N/100	Alive after 18 hours	
Acetate	N/10	3 hours	6.4
	N/100	Alive after 18 hours	

The prominent feature of these experiments is the marked resistance of amœbæ to the ammonium salts. The salts, with the exception of the hydroxide, produce an increase in viscosity of the protoplasm and death is accompanied by coagulation of the protoplasm. The toxicity of the hydroxide may be due to the alkalinity of the solution. Table I. gives a summary of the comparative toxicity of the ammonium salts to amœbæ. The time of death is only approximately correct for it is very difficult to determine the exact death point.

Injection Experiments.

The ammonium salts used in the immersion experiments were injected into amœbæ by means of Chambers' micromanipulator. The concentrations used were N/1 to N/100. The salts, with the exception of the carbonate, were non-lethal even in high concentrations (N/1) when injected into amœba in amounts equal to one fourth the volume of the cell. Injections of normal solutions of the hydroxide, chloride, phosphate, acetate and citrate result in a local elevation of the membrane in the form of a blister near the point of entrance of the pipette. The solutions rapidly diffused throughout the cell, producing a reversible gelation of the protoplasm. The animals gradually withdrew their pseudopodia and assumed a spherical form. Eventually, streaming of the protoplasm occurs, Brownian movement is resumed and the organism recovers. Usually one large pseudopodium is formed and the animal adopts a limax form. The rate of recovery depends upon the salt injected. Cells injected with the chloride and phosphate require a much longer time for recovery than those injected with citrate, acetate and hydroxide. The approximate rate of recovery is as follows: chloride > phosphate > citrate > acetate > hydroxide.

A normal solution of ammonium carbonate is lethal to amœbæ when injected in amount equal to the volume of the nucleus. Injections of N/1 or N/10 solutions of the carbonate result in an initial increase in viscosity, the animal withdraws its pseudopodia and becomes spherical; finally the cell membrane dissolves and the protoplasm remains as a gelatinous mass. The cell recovers from a dosage of N/100 ammonium carbonate.

Tearing the Plasma Membrane.

Amœbæ were immersed in N/10 and N/100 solutions of the above ammonium salts and the cell membrane torn with microdissection needles. If a small tear is made in the membrane a portion of the internal protoplasm escapes but the cell rapidly forms a new membrane over the injured surface. The rapidity of the formation of the membrane depends upon the salt used. More protoplasm escapes from a tear in the membrane when the cells are placed in the hydrate and carbonate than in the chloride, acetate, citrate or phosphate.

Discussion.

Ammonium salts hydrolyze to different degrees depending upon the acid radical which is combined with the ammonia. The entrance of ammonia into the cell from solutions of ammonium chloride has been studied by Jacobs (1922), who has conclusively shown that a cell (Rhodendron or starfish egg) may develop an intracellular alkalinity when placed in a solution of ammonium chloride which is decidedly acid. This change in internal pH is undoubtedly, as Jacobs concludes, due to the selective permeability of the cell membrane. Chambers (1922) has verified this conclusion by injecting ammonium chloride into starfish eggs, thereby producing an intracellular acidity which demonstrates that the selective permeability is confined to the membrane and not to the internal protoplasm.

Harvey (1911) has shown by using intracellular neutral red as an indicator that ammonia and its primary, secondary and tertiary alkyl substitution products enter cells with very little if any resistance and that death of the cell does not result from the intracellular alkalinity as is evident by the ability of the cell to recover when removed from the ammonia solution and placed in pure water. On the other hand, Harvey concludes that strong alkalies do not enter the cell until the surface is destroyed. The process is irreversible. He also states that the strong alkalies kill by affecting the plasma membrane and likewise on pages 534 and 547 he states that ammonia must affect the membrane since it produces changes in behavior similar to those produced by NaOH—vesicle formation, cessation of movement and finally death—but the

changes produced bear no relation to the speed of entrance. The results of the present experiments seem to confirm Harvey's conclusions that ammonia and certain ammonium salts exert their lethal effects on the plasma membrane and not on the internal protoplasm. The question may be raised that the non-toxicity of the injected salts is due to their outward diffusion from the cell. This is not probable for there is no reason to believe that the salts would diffuse out of the cell any faster than they would enter the cell. It may also be thought that if the toxicity of the ammonium salts is due to their actual passage—dissociated or undissociated—through the membrane or to a chemical combination between the salts and plasma membrane that the outward diffusion, if it occurs, from the injected cell would produce death. This, however, is not the case. So it may possibly be that the two sides of the membrane are different chemically or that the ammonium salts are adsorbed as molecules or ions on the external surface of the cell or they unite with some constituent of the outer surface of plasma membrane.

Summary.

A study was made by immersion and injection on the effects of the following ammonium salts: hydroxide, carbonate, chloride, phosphate, acetate and citrate on the protoplasm of *Amœba proteus* (?).

The effects of the ammonium salts are essentially due to the cations but may be modified by the anions.

The ammonium salts produce an increase in viscosity of the protoplasm in immersed amœbæ which is followed by a slight swelling of the protoplasm and disintegration of the cell. Injections of the salts, except the carbonate, into amœbæ produce a reversible increase in viscosity. The animals recover from dosages in amounts equal to one fourth the volume of the cell. Injection of the carbonate results in a disintegration of the cell.

When amœbæ are immersed in N/100 solutions of the salts and the cell membrane torn, a new membrane is formed over the injured surface.

These results seem to indicate that the toxicity of certain ammonium salts is due to their action on the plasma membrane and not on the internal protoplasm.

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